FKBP51: A Novel Target for Antidepressant Therapy

The present invention relates to a method of classifying an individual comprising analyzing the nucleic acid of a sample taken from said patient for nucleotide polymorphisms in a haplotype block comprising the gene encoding FKBP51 and/or determining the expression level of FKBP51 in said sample. In a preferred embodiment, the invention provides a method of predicting the response to antidepressant therapy. Furthermore, a method of developing an anti-depressant drug, and pharmaceutical compositions are provided.

In this specification, a number of documents is cited. The disclosure of these documents, including manufacturer's manuals, is herewith incorporated by reference in its entirety.

National surveys in Germany and in the United States agree that depression and other affective disorders (e.g. anxiety disorders and bipolar disorder) are among the most common medical conditions worldwide with lifetime prevalence for major depression reaching up to 14%. Pharmacotherapy is an effective treatment of depression and since the serendipitous discovery of the first antidepressant drug imipramine, a number of antidepressant drugs are now available. So far, all commercially available antidepressant drugs share the same pharmacological principle of enhancing monoaminergic neurotransmission, even though this may be achieved through a variety of mechanisms. Despite intensive efforts in the development of antidepressant drugs, major breakthroughs have only been achieved on the side effect profile of these drugs. Even though antidepressants are the most effective treatment for depressive disorders, there is still substantial need for improvement. Adequate therapy response to a single antidepressant is only

observed in 40-60% of patients, even when given in sufficiently high dose for enough time. There is also a substantial lag between the onset of treatment and clinical improvement that can last up to several weeks, even if "therapeutical" plasma concentrations can now be reached in a shorter amount of time. Furthermore, there are a percentage of patients that develop therapy-resistant depression, unresponsive to multiple treatment trials.

Although antidepressant drugs elicit quite divergent immediate effects, it is hypothesized that they all target a common final signaling pathway. The hypothalamic-pituitary-adrenal (HPA) system is considered to play a central pathophysiological role in this disease, because this system is not only consistently dysregulated in patients suffering from major depression, but normalization of this dyregulation is a prerequisite for successful treatment[1]. Currently used antidepressant drugs exert instant actions at various elements involved in monoaminergic neurotransmission[2]. These trigger a cascade of events that ultimately lead to the resolution of the clinical symptoms of depression. A host of data implicates central and peripheral disturbances of stress hormone regulation in the pathogenesis of depression and normalization of these defects as a necessary predecessor of clinical response to medication[3,4]. Central mechanisms for HPA-axis hyperactivity are an increased neurotransmission of the hypothalamic peptides corticotropin releasing hormone (CRH) and arginine vasopressin (AVP) that stimulate adrenocorticotropic hormone (ACTH) and cortisol release and an impaired negative feedback of this system due to glucocorticoid (GC) receptor (GR) insensitivity[5,6,7,8]. Preclinical and clinical studies suggest that one mechanism of action of antidepressant drugs may be to restore negative HPA-axis feedback via the GR leading to a downregulation of the overexpressed peptides CRH and AVP[9,10,11,12,13,14]. For proper functioning the GR, a ligand-activated transcription factor, depends on a large molecular complex that is necessary for proper receptor activation and transcriptional regulation of GR target genes[15,16].

A series of *in vivo* and *in vitro* studies have implicated FK506-binding protein 51 (FKBP51) as one among a host of physiologically relevant regulators of GR sensitivity. FKBP51 is part of the mature GR heterocomplex[19]. Upon hormone

binding, FKBP51 is replaced by FKBP52, which then recruits dynein into the complex, allowing its nuclear translocation and transcriptional activity[20]. A naturally occurring overexpression of FKBP51 appears to be the common cause of GR insensitivity in New world monkeys[21,22,23,26]. Moreover, overexpression of human FKBP51[21] *in vitro* clearly reduces hormone binding affinity and nuclear translocation of GR. Glucocorticoids induce FKBP51 mRNA expression via an ultra-short negative feedback loop for GR activity[24].

WO04/039837 relates to polynucleotides and polypeptides which can be used in the diagnosis and therapy of depression. Figure 4 of the application shows that FKPB51 (named in the application as FKPB5, FK506 binding protein 5, SEQ ID NO 32 or SEQ ID NO 33) is upregulated in transgenic animals overexpressing the corticotropin releasing hormone (CRH; see pages 4, 46-47). Furthermore, claims 1-25, 27, 29-31 relates to methods to diagnose a CRH induced gene expression profile by determining the expression or protein levels of FKBP51. WO2004/039837 does not however disclose any polymorphisms in FKBP51, nor does it make any association between polymorphisms in FKBP51 and improved response to antidepressants.

WO03/082210 relates to gene targets, polymorphisms, variants and methods for the diagnosis and treatment of schizophrenia. The document identifies four polymorphisms in FKBP51 (see pages 104 to 105 and Table 2) that are associated with schizophrenia. Two of these polymorphisms are known in the art (rs3777747 and rs992105). The application fails to provide, however, any implications for depression, let alone an association between polymorphisms in the FKBP51 locus and improved response to antidepressants.

It is known that the response to antidepressant therapy varies significantly from patient to patient. So far, these variations can only be determined and analysed in a retrospective manner. However, it would be highly desirable to classify individuals and to predict their response to therapy in order to tailor treatment to the individual patient's specific needs. The technical problem underlying the present invention therefore was to provide methods of classifying individuals.

The solution to said technical problem is achieved by providing the embodiments characterized in the claims.

Accordingly, the present invention relates to a method of classifying an individual comprising analyzing the nucleic acid of a sample taken from said individual for nucleotide polymorphisms in the gene encoding FKBP51 or in a haplotype block comprising the gene encoding FKBP51 and/or determining the expression level of FKBP51 in said sample. In other words, said analyzing is performed in vitro. In an alternative embodiment, said analyzing is performed ex vivo or in vivo.

The term "classifying" refers to the assignment of individuals to two or more groups or classes. In other words, individuals, previously unclassified, get labelled by their respective class. The assigned class label may refer to parameters used for classification, e.g. polymorphisms and/or expression levels, or may refer to parameters not used for classification because their values are not known beforehand, e.g. fast or slow response to therapy. In the first case, class discovery methods, e.g. clustering may be applied, whereas in the second case predictive classification methods are used. Classification may be done manually by a trained person or by a computer program provided with the values of the parameters used for class distinction. Patients have to give informed consent. Data have to be handled and kept secret in accordance with national laws.

The term "nucleotide polymorphism" refers to the occurrence of one or more different nucleotides or bases at a given location on a chromosome. Usually, polymorphisms are distinguished from mutations based on their prevalence. Sometimes a threshold of 1% prevalence in a population of individuals is considered for separating polymorphisms (more frequent) from mutations (less frequent). A single nucleotide polymorphism (SNP) is a polymorphism of a single nucleotide or base. The SNP database maintained at NCBI (http://www.ncbi.nlm.nih.gov/SNP/) divides SNPs into SNPs in the proximity of a known locus and such that are 5' further away than 2 kb from the most 5' feature of a gene and 3' further away than 500 bases from the most 3' feature of a gene. SNPs in the proximity of a known locus are further divided into SNPs occurring at an mRNA location and such that do not. SNPs occurring at an mRNA location comprise coding and non-coding SNPs.

In view of the evidence relating to nucleotide polymorphisms in a haplotype block comprising the gene encoding FKBP51 laid down in the present application, the inventors for the first time envisaged classifying individuals based on said polymorphisms.

Nucleotide polymorphisms may be associated or linked to a particular phenotype. Genetic screening exploits this association.

Genetic screening (also called genotyping or molecular screening), can be broadly defined as testing to determine if an individual has mutations, alleles or polymorphisms that either cause a specific phenotype or are "linked" to the mutation causing the phenotype. Linkage refers to the phenomenon that the DNA sequences which are close together in the genome have a tendency to be inherited together. Two or more sequences may be linked because of some selective advantage of co-inheritance. More typically, however, two or more polymorphic sequences are co-inherited because of the relative infrequency with which meiotic recombination events occur within the region between the two polymorphisms. The co-inherited polymorphic alleles are said to be in linkage disequilibrium (LD) with one another because, in a given population, they tend to either both occur together or else not occur at all in any particular member of the population. Indeed, where multiple polymorphisms in a given chromosomal region are found to be in linkage disequilibrium with one another, they define a quasi-stable genetic "haplotype", "haplotype block" or "LD block".

Location and size of haplotype blocks can be determined using computer programs, e.g. the program HaploBlockFinder[25]. Figure 1 shows a linkage disequilibrium map of the FKBP51 region. A haplotype block according to the invention extends, for example, from the polymorphism rs3800374 to rs4713921.

Furthermore, where a phenotype-causing polymorphism is found within a gene or haplotype or in linkage with a haplotype, one or more polymorphic alleles of the haplotype can be used as a diagnostic or prognostic indicator of the likelihood of developing a specific phenotype. Identification of a haplotype which spans or is

linked to a phenotype-causing polymorphism, serves as a predictive measure of an individual's likelihood of exhibiting that phenotype-causing polymorphism. Importantly, such prognostic or diagnostic procedures can be utilized without necessitating the identification and isolation of the actual phenotype-causing molecule. This is significant because the precise determination of the molecular basis of the establishment of a specific phenotype can be difficult and laborious, especially in the case of multifactorial phenotype.

Surprisingly, it has been found in healthy individuals that nucleotide polymorphisms in a haplotype block comprising the gene encoding FKBP51 correlate with the expression level of FKBP51. Therefore, according to the invention, the expression level of FKBP51 may be determined instead of or in addition to said nucleotide polymorphisms for the purpose of classifying individuals.

As stated above, there is an unmet need for methods of classifying patients suffering from depression with regard to their response to therapy. Unexpectedly, nucleotide polymorphisms in a haplotype block comprising the gene encoding FKBP51 were found to correlate significantly with the response to antidepressant therapy.

Therefore, it is preferred in accordance with the present invention that said classifying consists of or comprises predicting the response to therapy of an individual, wherein said individual is a patient suffering from depression. The availability of information as to the expected response to therapy is of great advantage for the patient, as a rational choice of drug and/or a tailored therapy regimen minimize adverse side effects and ensure the fastest decline of the symptoms of the disease.

Figure 2 shows a plot of the significance of the association with response to antidepressant therapy of SNPs in or in the proximity of the FKBP51 locus investigated by the inventors.

"Therapy" refers in accordance with the present invention to treatment with antidepressant drugs. Most antidepressant drugs target the transport of the neurotransmitters serotonin and/or norepinephrine, or the activity of the enzyme

monoamine oxidase. Common antidepressant drugs include: Selective serotonin-reuptake inhibitors (e.g., fluoxetine, paroxetine, sertraline, fluvoxamine), tricyclic antidepressants (e.g., amitriptyline, imipramine, desipramine, nortriptyline), monoamine oxidase inhibitors (e.g., phenelzine, isocarboxazid, tranylcypromine), and designer antidepressants such as mirtazapine, reboxetine, nefazodone. Benzodiazepines, or lithium carbonate may also be administered.

The inventors surprisingly found that elevated levels of FKBP51 correlate significantly with a faster response to therapy, i.e., the decline of symptoms of depression as determined by the Hamilton Depression Rating Scale (HAM-D) sets in earlier than in patients with lower levels of FKBP51. This observation is indeed unexpected, considering that transcriptional activity of GR heterocomplex requires that FKBP51 dissociates from the heterocomplex. The skilled person would expect that elevated levels of FKBP51 lead to an accumulation of the GR heterocomplex in the cytoplasm, thereby preventing GR from activating its target genes, the latter being considered essential for effective antidepressant therapy.

The authors of reference 24 (Vermeer et al., 2003) report a glucocorticoid-induced increase in lymphocytic FKBP51 mRNA expression and propose induction of FKBP51 mRNA as a marker for the assessment of individual GC sensitivity, for the in vitro measurement of GC potency, and the in vivo determination of GC bioavailability. While Vermeer et al. describe an induced increase of FKBP51 expression, the present invention relates to elevated levels of FKBP51 expression associated with nucleotide polymorphisms. Vermeer et al. do not refer to any polymorphisms in the FKBP51 locus. Furthermore, Vermeer et al. do not refer to any implications of their observations for the field of depression therapy.

Also reference 1 (Holsboer, 2000) discusses the implications of an altered FKBP51 level for corticosteroid signaling. According to Holsboer, ligand-bound GR associates with other proteins comprising heat shock proteins and immunophilins, specifically FKBP51 or FKBP52. An elevated FKBP51 level is discussed in relation to excessively high cortisol levels in squirrel monkeys[26], whereby it is speculated that the elevated FKBP51 level serves for compensating the notoriously high cortisol levels in squirrel monkeys.

Holsboer furthermore discusses possible causes of inherited glucocorticoid resistance, viz. a polymorphism in the GR gene or other alterations in genes, whose products are involved in glucocorticoid signaling. However, Holsboer does not designate any specific protein involved in glucocorticoid signaling.

The application of genotyping in the field of pharmacology gave rise to the fields of pharmacogenetics and, more recently, pharmacogenomics, the latter being used to denote studies correlating drug response with the analysis of multiple genes, e.g. their expression and/or polymorphisms or mutations in said genes. It is envisaged to stratify the population such that groups of individuals are obtained which receive tailored treatments. In order to get approval for tailored drugs and/or treatment regimens, the stratification of the population has to be extended to clinical trials.

Therefore, in a preferred embodiment, classifying according to the present invention consists of or comprises selecting an individual for a clinical trial. Individuals selected for clinical trials in accordance with the present invention may comprise healthy individuals and/or patients suffering from depression.

There are several published patent applications in the field of pharmacogenomics, that describe the use of gene sequence variations including SNPs (WO 00/50639, WO 99/64626) or expression profiling on a large scale for evaluating antidepressant and other drug response in patients. They give detailed technical descriptions on how to use SNPs to assess drug response. However, these documents neither anticipate nor suggest to investigate polymorphisms in the FKBP51 locus or FKBP51 expression levels.

It has been found by the inventors that nucleotide polymorphisms in a haplotype block comprising the gene encoding FKBP51 correlate with the number of a patient's previous episodes of depression. Therefore, said polymorphisms and/or the expression level of FKBP51 can be used as (a) prognostic marker/s for an elevated number of episodes of depression.

Accordingly, in a further preferred embodiment, classifying according to the present invention consists of or comprises predicting the predisposition of an individual for an

elevated number of episodes of depression, wherein said individual is a patient suffering from depression.

In a preferred embodiment, the nucleic acid to be analyzed is genomic DNA (gDNA).

In a more preferred embodiment, the haplotype block comprises at least one SNP selected from the group consisting of rs2766534, rs4711429, rs4713921, rs9462104, rs9394312, rs4713916, rs943297, rs9380528, FKBP5UT5A, FKBP5UT5C, rs9380526, rs10947563, rs9380525, rs6912833, rs2143404, rs1360780, rs1591365, rs7748266, rs9470069, rs6926133, rs3777747, rs4713899, rs2395634, rs7753746, rs3800373, rs10807151, rs3800374, rs9348978, rs11751447 and, rs2395631. According to this embodiment, the identification and analysis of any nucleotide polymorphism in said haplotype block is envisaged.

The identifiers recited above permit retrieval of sequence and other information for the respective SNP in the SNP database maintained at the NCBI (http://www.ncbi.nlm.nih.gov/SNP/). Obviously, this does not apply for the novel SNPs according to the invention, i.e. FKBP5UT5A and FKBP5UT5C.

In a yet more preferred embodiment, the polymorphism is a SNP in a non-coding region of said gene or haplotype block.

Most preferred, the polymorphism is at least one SNP selected from the group consisting of rs2766534, rs4711429, rs4713921, rs9462104, rs9394312, rs4713916, rs943297, rs9380528, FKBP5UT5A, FKBP5UT5C, rs9380526, rs10947563, rs9380525, rs6912833, rs2143404, rs1360780, rs1591365, rs7748266, rs9470069, rs6926133, rs3777747, rs4713899, rs2395634, rs7753746, rs3800373, rs10807151, rs3800374, rs9348978, rs11751447 and, rs2395631.

These 30 SNPs are most significantly associated with improved response to antidepressant treatment. The following table summarises the position of these SNPs on chromosome 6, the region of the FKBP51 locus containing the SNP, nucleotide changes for each genotype, heterozygote frequency and the p-value for an association with fast response to an antidepressant drug.

Table 1

SNP	Position On Chr 6	type	Nucleotide change	Heterozygote frequency	p-value
rs2766534	35732569	Promoter	G/T	33,2%	0,0057
rs4711429	35730403	Promoter	G/A	38,3%	0,0076
rs4713921	35728632	Promoter	C/T	39,1%	0,0036
rs9462104	35721700	Promoter	T/C	38,5%	0,0043
rs9394312	35719185	Promoter	C/G	49%	0,0016
rs4713916	35716838	Promoter	G/A	36.6%	5.5×10^{-5}
rs943297	35714715	Promoter	G/A	38.8%	3.1×10^{-4}
rs9380528	35711107	Promoter	A/G	49%	0,0034
FKBP5UT5A	35706425	Promoter	G/C	0.3%	*
FKBP5UT5C	35705284	Promoter	A/G	1.6%	*
rs9380526	35705182	Promoter	T/C	41.4%	4.8×10^{-4}
rs10947563	35700292	Intron 1	A/G	35.8%	0,0013
rs9380525	35679893	Intron 1	C/G	42.1%	2.7×10^{-4}
rs6912833	35664440	Intron 1	T/A	44.8%	3.5×10^{-4}
rs2143404	35657536	Intron 2	C/T	24.8%	0,0460
rs1360780	35654426	Intron 2	C/T	40.1%	1.2×10^{-4}
rs1591365	35650962	Intron 3	A/G	38.6%	6.2×10^{-4}
rs7748266	35639599	Intron 3	C/T	26,4%	0,0348
rs9470069	35629516	Intron 5	C/G	18,4%	0,0450
rs6926133	35626230	Intron 5	A/C	27,9%	0,0135
rs3777747	35625857	Intron 5	A/G	49,6%	0,0061
rs4713899	35616136	Intron 5	A/G	25,4%	0,0244
rs2395634	35614615	Intron 5	A/G	39,9%	0,0036
rs7753746	35612277	Intron 5	A/G	26,1%	0,0328
rs3800373	35589331	3'UTR	A/C	33.0%	2.8×10^{-5}
rs10807151	35587723	3' of FKBP51	T/C	25,9%	0,0125
rs3800374	35584261	3' of	C/T	26,7%	0,0354

		FKBP51			
rs9348978	35564135	3' of FKBP51	G/A	48.2%	5.8×10^{-4}
rs11751447	35556977	3' of FKBP51	T/C	48%	0,0179
rs2395631	35554170	3' of FKBP51	G/A	47,2%	0,0114

* Patient sample too small to estimate p-value

Following the identification of individuals homozygous for the TT allele of rs2766534, or the AA allele of rs4711429, or the TT allele of rs4713921, or the CC allele of rs9462104, or the GG allele of rs9394312, or the AA allele of rs4713916, or the AA allele of rs943297, or the GG allele of rs9380528, or the CC allele of rs9380526, or the GG allele of rs10947563, or the GG allele of rs9380525, or the AA allele of rs6912833, or the TT allele of rs2143404, or the TT allele of rs1360780, or the GG allele of rs1591365, or the TT allele of rs7748266, or the GG allele of rs9470069, or the CC allele of rs6926133, or the GG allele of rs3777747, or the GG allele of rs4713899, or the GG allele of rs2395634, or the GG allele of rs7753746, or the CC allele of rs3800373, or the CC allele of rs10807151, or the TT allele of rs3800374, or the AA allele of rs9348978, or the CC allele of rs11751447, or the AA allele of rs2395631, or individuals with the CC, CG, or GG allele of FKBP5UT5A, or individuals with the GG, GA, or AA allele of FKBP5UT5C, or patients with an increase in the expression or protein levels of FKBP51, a normal prudent physician would recommend prescription or administration of an antidepressant drug. Administration and dosage of antidepressant drugs can vary between patients and are well known in the medical art, see, for example Benkert and Hippius, "Kompendium der Psychiatrischen Pharmakotherapie", Springer Verlag Publishing, 2000; Albers, "Handbook of Psychiatric Drugs: 2001-2002 Edition", Current Clinical Strategies Publishing, 2000. Preferred examples include between 5 mg and 80 mg per day, preferably 20 mg, fluoxetine; between 5 mg and 50 mg per day, preferably 20 mg, paroxetine; between 5 mg and 200 mg per day, preferably 50 mg, sertraline; between 5 mg and 300 mg per day, preferably 100 mg, fluvoxamine; between 5 mg and 100 mg per day, preferably 30 mg, mirtazapine; between 4 mg and 50 mg, preferably 8 mg, reboxetine; between 5 mg and 600 mg per day, preferably 200 mg,

nefazodone; between 450 mg and 1800 mg per day, preferably 900 mg, lithium carbonate.

Furthermore, the SNPs listed in Table 1 correlate with the number of previous episodes of depression. This is exemplified by the data shown in Figures 5 (rs1360780), 6 (rs4713916) and 7 (rs3800373).

Several methods of analyzing a nucleic acid for nucleotide polymorphisms are known in the art. Accordingly, in a preferred embodiment, analyzing the nucleic acid by the method of the invention comprises (a) a primer extension assay; (b) a differential hybridization assay; and/or (c) an assay which detects allele-specific enzyme cleavage.
The underlying principles and the use of said assays has been described in an article of Asil Memisoglu (www.thebiotechclub.org/industry/emerging/pharmacogenomics.php). Examples for said assays are known by a person skilled in the art.

In a more preferred embodiment, the method according to the invention comprises, prior to analyzing, amplification of at least a portion of said gene or haplotype block. Preferably, said amplification is effected by or said amplification is the polymerase chain reaction (PCR). The principles of and procedures to perform PCR are known in the art.

In a more preferred embodiment, said amplification reaction uses primers which hybridize specifically with a portion of said gene or haplotype block. Means of ensuring specificity of hybridization according to the present invention are known in the art and include stringent hybridization conditions. The term "stringent hybridization conditions", as used in the description of the present invention, is well known to the skilled artisan. Appropriate stringent hybridization conditions for each sequence may be established by a person skilled in the art on well-known parameters such as temperature, composition of the nucleic acid molecules, salt conditions etc.; see, for example, Sambrook et al., "Molecular Cloning, A Laboratory Manual"; CSH Press, Cold Spring Harbor, 1989 or Higgins and Hames (eds.), "Nucleic acid hybridization, a practical approach", IRL Press, Oxford 1985, see in

particular the chapter "Hybridization Strategy" by Britten & Davidson, 3 to 15. Stringent hybridization conditions are, for example, conditions comprising overnight incubation at 42° C in a solution comprising: 50% formamide, 5x SSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°. Other stringent hybridization conditions are for example 0.2 x SSC (0.03 M NaCl, 0.003M Natriumcitrat, pH 7) at 65°C.

In a preferred embodiment, the primers to be used for said amplification reaction have the sequence as set forth in SEQ ID NOs: 21 and 22; 23 and 24; 25 and 26; 27 and 28; 29 and 30; 1 and 2; ; 31 and 32; 33 and 34; 35 and 36; 37 and 38; 39 and 40; 41 and 42; 43 and 44; 45 and 46; 47 and 48; 5 and 6; 49 and 50; 51 and 52; 53 and 54; 55 and 56; 57 and 58; 59 and 60; 61 and 62; 63 and 64; 7 and 8; 65 and 66; 67 and 68; 69 and 70; 71 and 72; or 73 and 74 and disclosed in the following table.

Table 2

SNP-ID	sense	antisense
rs2766534	ACGTTGGATGTCGAAGGGACTTATT CCTCC (SEQ ID NO: 21)	ACGTTGGATGCAGCAGAAGGAAGAC ATCAG (SEQ ID NO: 22)
rs4711429	ACGTTGGATGACACACGGCTCATCT GTAAC (SEQ ID NO: 23)	ACGTTGGATGATGCCAGGCATTTGG GTTTC (SEQ ID NO: 24)
rs4713921	ACGTTGGATGAAGCCCTGTGGTTTT ATGCC (SEQ ID NO: 25)	ACGTTGGATGTGGAACAATTCTGTC CCCAG (SEQ ID NO: 26)
rs9462104	ACGTTGGATGTTTCCTCCCTAGATTC CCAG (SEQ ID NO: 27)	ACGTTGGATGGTCCCAGTGGAACTT TATGG (SEQ ID NO: 28)
rs9394312	ACGTTGGATGACAAGCTATCGGGCT TTCTC (SEQ ID NO: 29)	ACGTTGGATGAGACAATCTCCCAGT TGCAG (SEQ ID NO: 30)
rs4713916	ACGTTGGATGTATCTGGCAACCCTA ACCTC (SEQ ID NO: 1)	ACGTTGGATGCCTAACGAGATAGTG AGGAG (SEQ ID NO: 2)
rs943297	ACGTTGGATGTCTGAAATCCAGCTG GACAC (SEQ ID NO: 31)	ACGTTGGATGAGTGAGACTCTGTCC AAAAG (SEQ ID NO: 32)
rs9380528	ACGTTGGATGTTGAGACCAGATGTT GGAGG (SEQ ID NO: 33)	ACGTTGGATGGACAGAGTCTGGCTC TGTTG (SEQ ID NO: 34)
FKBP5UT5A	ACGTTGGATGTACAGACCCTGCAAA	ACGTTGGATGTGCGCTATGGCTGCA

	GAACC (SEQ ID NO: 35)	GATAC (SEQ ID NO: 36)
FKBP5UT5C	ACGTTGGATGTACAGACCCTGCAAA GAACC (SEQ ID NO: 37)	ACGTTGGATGTGCGCTATGGCTGCA GATAC (SEQ ID NO: 38)
rs9380526	ACGTTGGATGAAACAGGGAAGCTTC TAGGC (SEQ ID NO: 39)	ACGTTGGATGAAGCCAAGACTGAAA CCGCC (SEQ ID NO: 40)
rs10947563	ACGTTGGATGTATTACAGGCGTGAG CCATC (SEQ ID NO: 41)	ACGTTGGATGATGGTCTTTAGAGGA ATTCT (SEQ ID NO: 42)
rs9380525	ACGTTGGATGGACAGAGTGAGACTC CTCTA (SEQ ID NO: 43)	ACGTTGGATGTTCTCAAACCAGCAC TCAGG (SEQ ID NO: 44)
rs6912833	ACGTTGGATGGGGAAAAGAATCAAG GGAAG (SEQ ID NO: 45)	ACGTTGGATGCTGGAACTAAGTGAT CTGTG (SEQ ID NO: 46)
rs2143404	ACGTTGGATGGGTTAGGTAGAGCTC AGTTC (SEQ ID NO: 47)	ACGTTGGATGGTAGAGAACCTGGTA AGAAG (SEQ ID NO: 48)
rs1360780	ACGTTGGATGAAGAGATCCAGGCAC AGAAG (SEQ ID NO: 5)	ACGTTGGATGTGCCAGCAGTAGCAA GTAAG (SEQ ID NO: 6)
rs1591365	ACGTTGGATGGTGGCAAATAGGAGT TCTCC (SEQ ID NO: 49)	ACGTTGGATGTTGGCAGGTGTTTTT CTGAG (SEQ ID NO: 50)
rs7748266	ACGTTGGATGAGACCACACCACAGA ACAAG (SEQ ID NO: 51)	ACGTTGGATGTTGCTTAACCCTCTC CAGAC (SEQ ID NO: 52)
rs9470069	ACGTTGGATGATATACATACAGGCT GGCCG (SEQ ID NO: 53)	ACGTTGGATGACTCCTGACCTCGTG ATCTG (SEQ ID NO: 54)
rs6926133	ACGTTGGATGGATGACTGATACTTC AGTCT (SEQ ID NO: 55)	ACGTTGGATGGAAAGCAAAGCTGAA AAGTAG (SEQ ID NO: 56)
rs3777747	ACGTTGGATGCCACTCTTACATTCCT CTCC (SEQ ID NO: 57)	ACGTTGGATGTCCCTCTCCAAATCT CACTG (SEQ ID NO: 58)
rs4713899	ACGTTGGATGAGTGGAGCTATAGGA GCTAG (SEQ ID NO: 59)	ACGTTGGATGACGGAAAGACTGCTG ATTGC (SEQ ID NO: 60)
rs2395634	ACGTTGGATGTCGAAGGGACTTATT CCTCC (SEQ ID NO: 61)	ACGTTGGATGCAGCAGAAGGAAGAC ATCAG (SEQ ID NO: 62)
rs7753746	ACGTTGGATGAGAATAAACCTGTGT TTCTG (SEQ ID NO: 63)	ACGTTGGATGAGGAGCAACCATATT TCTAA (SEQ ID NO: 64)
rs3800373	ACGTTGGATGAAACCCCTAGTGTAG AAGAG (SEQ ID NO: 7)	ACGTTGGATGTTTACACTCCTCTATC ATGC (SEQ ID NO: 8)
rs10807151	ACGTTGGATGGGGTGAAAGTGGCA GAACAA (SEQ ID NO: 65)	ACGTTGGATGCCGCTGGCTAATGAA AAAAC (SEQ ID NO: 66)
rs3800374	ACGTTGGATGAGACGATGGACCCAT TTTAC (SEQ ID NO: 67)	ACGTTGGATGTCTTTTCCAAGTGGT GAACC (SEQ ID NO: 68
rs9348978	ACGTTGGATGAATTTTTACCACTGAG CAGG (SEQ ID NO: 69)	ACGTTGGATGGCTTTGGCTTTACGG AAGAG (SEQ ID NO: 70)

rs11751447	ACGTTGGATGATTCTCTCTTGAGTC GGTGC (SEQ ID NO: 71)	ACGTTGGATGTAGAACCTTGCCACA GAGAC (SEQ ID NO: 72)
rs2395631	ACGTTGGATGTGCAAAAACTAACAA AAGCC (SEQ ID NO: 73)	ACGTTGGATGAGGGTTATCAACCTT GAGGC (SEQ ID NO: 74)

In the primer extension assay according to the invention the target sequence is annealed to a primer complementary to the region adjacent to the SNP site. Dideoxyribonucleotides (ddNTPS) and DNA polymerase are added to the mixture and the primer is extended by a single nucleotide. The single nucleotide added is dependent on the allele of the amplified DNA. Primer extension biochemistry can be coupled with a variety of detection schemes, comprising fluorescence, fluorescence polarization (FP), luminescence and mass spectrometry (MS).

Accordingly, in a preferred embodiment of the method of the invention, the primer extension assay uses a primer which hybridizes specifically with a portion of said gene or haplotype block which is adjacent to a polymorphism. The following table discloses the primers used for genotyping using single-base extension assays.

Table 3

SNP-ID	Extension primer
rs2766534	CCGCCCTACACTTTCAC (SEQ ID NO : 75)
rs4711429	ATCTGTAACTTAGAGCCCTT (SEQ ID NO : 76)
rs4713921	CAGTTGAAAGAGCCTCAC (SEQ ID NO : 77)
rs9462104	CCTTAGGCATAACCACC (SEQ ID NO : 78)
rs9394312	TTCTCCCGAGGCTCCCA (SEQ ID NO : 79)
rs4713916	GACTCCTACATTTTCCTCT (SEQ ID NO : 9)
rs943297	CTGGACACAATTCCTTAGTTA (SEQ ID NO : 80)
rs9380528	AACTGAGATCATGCCACT (SEQ ID NO : 81)
FKBP5UT5A	GGAAAGGGCTGCTCATCC (SEQ ID NO : 82)
FKBP5UT5C	TCATTAACGCAGAAAAACAAA (SEQ ID NO : 83)
rs9380526	GAGGACAGACAAATGACT (SEQ ID NO : 84)

rs10947563	CAGTAATATGCTTGCTGTACC (SEQ ID NO : 85)
rs9380525	TTACTAGTGACATTCTAAAGGAG (SEQ ID NO : 86)
rs6912833	GATGCAAGAATAGCATGGATC (SEQ ID NO : 87)
rs2143404	GAGCTCAGTTCCCAGGG (SEQ ID NO : 88)
rs1360780	GGCTTTCACATAAGCAAAGTTA (SEQ ID NO : 11)
rs1591365	TGTCAGTTGTTTTTCCTTGAA (SEQ ID NO : 89)
rs7748266	GAACAAGAAACCAGTTGTATCA (SEQ ID NO : 90)
rs9470069	CTGTAATTCCAGCACTTTG (SEQ ID NO : 91)
rs6926133	AGTCTTTAAGTTTAATTGCAGGTC (SEQ ID NO : 92)
rs3777747	ATTCCTCTCCTTTCCAGC (SEQ ID NO : 93)
rs4713899	AGGCTCATCTGCATCTGTTAC (SEQ ID NO : 94)
rs2395634	TAGAATGTTCTTACACAAATCTAG (SEQ ID NO : 95)
rs7753746	GTGTTTCTGGTTTGTTACC (SEQ ID NO : 96)
rs3800373	AAGAGCAACTATTTATTTGTCAAC (SEQ ID NO : 12)
rs10807151	TGGCAGAACAATGCATC (SEQ ID NO : 97)
rs3800374	CCCATTTTACAGTTATGGGCTC (SEQ ID NO : 98)
rs9348978	CTGAGCAGGTGAAAAAT (SEQ ID NO : 99)
rs11751447	GGTGCCTCGTGCAGAGC (SEQ ID NO : 100)
rs2395631	TTTCATCTGATAGGCCCAAC (SEQ ID NO : 101)

Therefore, in a preferred embodiment of the method of the invention, the primer to be used for said primer extension assay has the sequence as set forth in SEQ ID NO: 75, 76, 77, 78, 79, 9, 80, 81, 82, 83, 84, 85, 86, 87, 88, 11, 89, 90, 91, 92, 93, 94, 95, 96, 12, 97, 98, 99, 100, or 101.

Further methods of analyzing a nucleic acid for the occurrence of polymorphisms are differential hybridization and allele-specific enzyme cleavage. The latter is also referred to as Restriction Fragment Length Polymorphism (RFLP).

Restriction Fragment Length Polymorphism (RFLP) is a technique in which species, strains, or, as envisaged by the inventors, polymorphic alleles may be differentiated by analysis of patterns derived from cleavage of their DNA. If two organisms differ in

the distance between sites of cleavage of a particular restriction endonuclease, the length of the fragments produced will differ when the DNA is digested with a restriction enzyme.

In a preferred embodiment, the differential hybridization assay according to the invention or said assay detecting allele-specific enzyme cleavage according to the invention uses probes which hybridize specifically with a portion of said gene or haplotype block which comprises a polymorphism. The detection of allele-specific enzyme cleavage comprises (a) hybridizing said probes, preferably under stringent conditions, to nucleic acid molecules comprised in a sample taken from an individual; (b) digesting the product of said hybridization with a restriction endonuclease; and (c) analyzing the product of said digestion.

In a more preferred embodiment, said probes have the sequence as set forth in SEQ ID NO: 102 and 103; 104 and 105; 106 and 107; 108 and 109; 110 and 111; 13 and 14; 112 and 113; 114 and 115; 116 and 117; 118 and 119; 120 and 121; 122 and 123; 124 and 125; 126 and 127; 128 and 129; 17 and 18; 130 and 131; 132 and 133; 134 and 135; 136 and 137; 138 and 139; 140 and 141; 142 and 143; 144 and 145; 19 and 20; 146 and 147; 148 and 149; 150 and 151; 152 and 153; or 154 and 155. The sequences are shown in the table below.

Table 4

SNP	**Column A** Probe for allele associated with improved response when present in a homozygous state	**Column B** Probe for allele associated with inferior response when present in a patient
rs2766534	TTGAAAAGGGGCATGTGAAAGTGTAGGGC (SEQ ID NO: 102)	TTGAAAAGGGGCAGGTGAAAGTGTAGGGC (SEQ ID NO: 103)
rs4711429	GGGAGCCTGTAAAGGGCTCTAAGTT (SEQ ID NO: 104)	GGGAGCCTGTGAAGGGCTCTAAGTT (SEQ ID NO: 105)
rs4713921	TCCACTGAGATGTGAGGCTCTT (SEQ ID NO: 106)	TCCACTGAGACGTGAGGCTCTT (SEQ ID NO: 107)
rs9462104	AGAAACTCTTAACGGTGGTTATG (SEQ ID NO: 108)	AGAAACTCTTAATGGTGGTTATG (SEQ ID NO: 109)

rs9394312	CACCAAGATCAGTGGGAGCCTCG (SEQ ID NO: 110)	CACCAAGATCACTGGGAGCCTCG (SEQ ID NO: 111)
rs4713916	ATTTTCCTCTATCTTGGTCCA (SEQ ID NO: 13)	ATTTTCCTCTGTCTTGGTCCA (SEQ ID NO: 14)
rs943297	ATTCCTTAGTTAAGTCCTTGTTCTT (SEQ ID NO: 112)	ATTCCTTAGTTAGGTCCTTGTTCTT (SEQ ID NO: 113)
rs9380528	GATCATGCCACTGTACTCCAGCC (SEQ ID NO: 114)	GATCATGCCACTATACTCCAGCC (SEQ ID NO: 115)
FKBP5UT5A	ACCTGGATGTCGGATGAGCAGC (SEQ ID NO: 116)	ACCTGGATGTGGGATGAGCAGC (SEQ ID NO: 117)
FKBP5UT5C	AGAAAAACAAAGTTGATCAAAAT (SEQ ID NO: 118)	AGAAAAACAAAATTGATCAAAAT (SEQ ID NO: 119)
rs9380526	GGTTCTGTCTCAGTCATTTGTCT (SEQ ID NO: 120)	GGTTCTGTCTTAGTCATTTGTCT (SEQ ID NO: 121)
rs10947563	TCTTCATGCCGGGTACAGC (SEQ ID NO: 122)	TCTTCATGCCAGGTACAGC (SEQ ID NO: 123)
rs9380525	CAGGGTCTTCTGCTCCTTTAG (SEQ ID NO: 124)	CAGGGTCTTCTCCTCCTTTAG (SEQ ID NO: 125)
rs6912833	TAGCATGGATCAGTTCATCAACTA (SEQ ID NO: 126)	TAGCATGGATCTGTTCATCAACTA (SEQ ID NO: 127)
rs2143404	GAATCTTGAATGTCCCTGGGAACTG (SEQ ID NO: 128)	GAATCTTGAATGCCCCTGGGAACTG (SEQ ID NO: 129)
rs1360780	AGCAAAGTTATACAAAACAAA (SEQ ID NO: 17)	AGCAAAGTTACACAAAACAAA (SEQ ID NO: 18)
rs1591365	TTTTTCCTTGAAGTGACAGACTTA (SEQ ID NO: 130)	TTTTTCCTTGAAATGACAGACTTA (SEQ ID NO: 131)
rs7748266	GTTGTATCATTCAGTATATAT (SEQ ID NO: 132)	GTTGTATCACTCAGTATATAT (SEQ ID NO: 133)
rs9470069	TCCAGCACTTTGGGAGGCCAAGG (SEQ ID NO: 134)	TCCAGCACTTTGCGAGGCCAAGG (SEQ ID NO: 135)
rs6926133	AATGTAGCCACGACCTGCAATT (SEQ ID NO: 136)	AATGTAGCCAAGACCTGCAATT (SEQ ID NO: 137)
rs3777747	CTCCTTTCCAGCGCTATTATTG (SEQ ID NO: 138)	CTCCTTTCCAGCACTATTATTG (SEQ ID NO: 139)
rs4713899	GCCCTGATTGCGGTAACAGATG (SEQ ID NO: 140)	GCCCTGATTGCAGTAACAGATG (SEQ ID NO: 141)
rs2395634	CACAAATCTAGGTGGAACAGCCT (SEQ ID NO: 142)	CACAAATCTAGATGGAACAGCCT (SEQ ID NO: 143)
rs7753746	GACAGTTTAGTGGGTAACAAACCA (SEQ ID NO: 144)	GACAGTTTAGTAGGTAACAAACCA (SEQ ID NO: 145)

rs3800373	ATTTGTCAACCCTACAGATTT (SEQ ID NO: 19)	ATTTGTCAACACTACAGATTT (SEQ ID NO: 20)
rs10807151	TTTTTAGCAACGATGCATTGTTC (SEQ ID NO: 146)	TTTTTAGCAATGATGCATTGTTC (SEQ ID NO: 147)
rs3800374	TATGGGCTCTCAAAATTCA (SEQ ID NO: 148)	TATGGGCTCCCAAAATTCA (SEQ ID NO: 149)
rs9348978	ATACTTATTTGAATTTTTCACCT (SEQ ID NO: 150)	ATACTTATTTGGATTTTTCACCT (SEQ ID NO: 151)
rs11751447	CTCGTGCAGAGCCCCTCCTCTGCTG (SEQ ID NO: 152)	CTCGTGCAGAGCTCCTCCTCTGCTG (SEQ ID NO: 153)
rs2395631	AGGCCCAACAGTATACCTTTGC (SEQ ID NO: 154)	AGGCCCAACGGTATACCTTTGC (SEQ ID NO: 155)

Each of these probes recognizes one of the two alleles at the respective SNPs only. For example, in a hybridization experiment for the rs4713916 SNP, a hybridization signal with the probe in column A and no signal with the probe in column B would be indicative of a person with improved response to therapy, whilst a signal with both probes and with only the probe from column B would be indicative of inferior response. The same also applies to the other SNPs (rs2766534, rs4711429, rs4713921, rs9462104, rs9394312, rs943297, rs9380528, FKBP5UT5A, FKBP5UT5C, rs9380526, rs10947563, rs9380525, rs6912833, rs2143404, rs1360780, rs1591365, rs7748266, rs9470069, rs6926133, rs3777747, rs4713899, rs2395634, rs7753746, rs3800373, rs10807151, rs3800374, rs9348978, rs11751447 and, rs2395631).

In a more preferred embodiment, the probes used for the differential hybridization assay are immobilized on a supporting material. Probe sets, immobilized on a filter or solid support and arranged in arrayed form, are generally referred to as microarrays or DNA chips.

As described above, SNPs which correlate with response to therapy in patients suffering from depression, correlate also with FKBP51 expression levels. The latter correlation has been observed in healthy individuals. Therefore, the administration of antidepressant drugs does not account for the observed differences in FKBP51 expression level. Without being bound by a specific theory, it is assumed that SNPs in the FKBP51 locus are responsible for different FKBP51 expression levels.

Therefore, the use of FKBP51 expression levels for classification and prognostic methods according to the invention is envisaged.

In a preferred embodiment, the FKBP51 expression level to be determined is the mRNA expression level. Methods for the determination of mRNA expression levels are known in the art and comprise Real Time RT-PCR, Northern blotting and hybridization on microarrays or DNA chips equipped with one or more probes or probe sets specific for FKBP51 transcripts.

In a further preferred embodiment, the expression level to be determined is the protein expression level. The skilled person is aware of methods for the quantitation of proteins. Amounts of purified protein in solution can be determined by physical methods, e.g. photometry. Methods of quantifying a particular protein in a mixture rely on specific binding, e.g of antibodies. Specific detection and quantitation methods exploiting the specificity of antibodies comprise immunohistochemistry (in situ) and surface plasmon resonance. Western blotting combines separation of a mixture of proteins by electrophoresis and specific detection with antibodies. Example 1, sub-heading "Quantification of FKBP51 protein levels in lymphocytes", provides an example of how protein expression levels can be determined according to the invention. Example 4 and Figure 9 present and discuss results obtained by Western blotting and their implications for the methods according to the invention.

In a preferred embodiment, the method of selecting an individual for a clinical trial according to the invention, further comprises the steps: (a) identifying a compound modulating the activity of FKBP51; and optionally (b) optimizing the pharmacological properties of the compound identified in (a); and (c) performing said clinical trials; wherein said clinical trials are clinical trials of the compound identified in (a) or optimized in (b). In this embodiment, the method of the invention may be used for the development of an antidepressant drug.

In principle, and regarding step (a), to change the activity of FKBP51 either protein level or protein function could be altered. To change protein level either promoter activity or protein stability could be altered.

In a preferred embodiment, the compound modulating the activity of FKBP51 is an activator of FKBP51.

In a more preferred embodiment, the compound modulating the activity of FKBP51 increases the expression of FKBP51. The screen described below exemplifies the method for the identification of such compounds.

Screen for compounds that alter the promoter activity of FKBP51

The screen may employ any of the assays described below.
Reporter gene assays employing transient transfection into various cells from yeast to mammals is a standard procedure. For FKBP51, the regulatory regions, i.e. gene promoter and enhancer have to be cloned upstream of the structural part of a gene encoding for a reporter (cf. e.g. Mühlhardt, Der Experimentator: Molekularbiologie, Gustav Fischer Verlag 1999), for example, β-galactosidase, firefly luciferase, renilla luciferase, a fluorescing protein (e.g. GFP, EGFP, EYFP etc.), human growth hormone, CAT (chloramphenicolacetyltransferase), TAT (tyrosylaminotransferase), alkaline phosphatase including SEAP, and peroxidase. Suitable eukaryotic cell lines include immortalized tumour cell lines, listed for example in the catalogues of ATCC and ETCC, in particular the cell lines HeLa, HEK, L929, NIH3T3, COS1, COS7, HepG2, H4-II-E-C3, Saos, K562, SK-N-MC, HT22, CV-1 preferably SK-N-MC or HEK cells. As an alternative to transient transfection assays, an in vitro transcription/translation system may be adapted, or stably transfected cell lines may be used accordingly. A number of methods are established to generate cell lines stably transfected with a reporter gene plasmid.

All assays have to be performed in the presence or absence of test compounds that comprise small molecules, peptides, aptamers and antibodies or fragments or derivatives thereof. An increase in expression of FKBP51 in the presence of a test compound identifies the compound as a hit.

This screen can be extended to modulators of the expression of FKBP52 by replacing the FKBP51 nucleic acid or amino acid sequence with the FKBP52 nucleic acid or amino acid sequence.

In a further preferred embodiment, the compound modulating the activity of FKBP51 enhances function and/or stability of FKBP51. The screen described below exemplifies the method for the identification of such compounds.

Screen for compounds that bind to FKBP51 and change its function or stability

This screen is designed to first identify compounds that bind to FKBP51 and subsequently determine FKBP51-binding compounds that in addition have functional effects.

For the purpose of identifying FKBP51-binding compounds, for example a phage display library may be used. While using a phage display library allows to screen peptide compounds, the Biacore 3000 system is suitable for detection of any compound that binds to FKBP51 larger than 180 dalton. The Biacore system requires purified, human FKBP51, which can be from any source, preferably expressed in a bacterial expression system, preferably as a His-tagged protein. Purification of His-tagged protein is an established, standardized procedure and can be performed according to the manufacturers recommendation, e.g. Qiagen or Invitrogen. Any expression vector for recombinant proteins in bacteria can be used, preferably one that provides inducible expression, preferably the vector series pProExHTa (Invitrogen).

Any technique that attaches the purified FKBP51 protein to the Biacore sensor chip surface may be used. In particular, the use of NTA sensor chips is suitable for the His-tagged version of the recombinant FKBP51. Automatic screening of a large number of compounds can be performed according to established protocols by the Biacore manufacturer. Compounds that bind to FKBP51 will then be subjected to subsequent analyses to identify those with an effect on protein stability of FKBP51, on hormone binding of GR or on GR-dependent transcription.

For protein stability, mammalian cell lines like HeLa, HEK, L929, NIH3T3, COS1, COS7, HepG2, H4-II-E-C3, Saos, K562, SK-N-MC, HT22, CV-1 preferably SK-N-MC or HEK cells, are treated for different time intervals from 0h to 48h, eventually up to

several weeks with the compound and levels FKBP51 are assessed by standard western blotting techniques on an Imaging System, preferably an imaging station like Kodak 440CF.

To determine hormone binding affinity, a number of different protocols are available. Binding affinity can be measured in tissues, in cell extracts, or in a partially purified extract of the glucocorticoid receptor, which needs to be supplemented with recombinant FKBP51. A recombinant, partially purified human glucocorticoid receptor expressed in the Baculovirus system is available from Panvera.

Assays for determining glucocorticoid receptor-dependent gene transcription are well known in the art, see for example Herr et al., Mol.Pharmacol. 57 (2000), 732-737; Hollenberg et al., Cancer Res. 49 (1989), 2292s-2294s; Kullmann et al., J.Biol.Chem. 273 (1998), 14620-14625; Rupprecht et al., Eur. J. Pharmacol. 247 (1993), 145-154. These assays may be performed using one of the transient transfection methods or in stably transfected cell lines.

This screen can be extended to FKBP52 binders and modulators by replacing the FKBP51 nucleic acid or amino acid sequence with the FKBP52 nucleic acid or amino acid sequence.

Methods for the optimization of the pharmacological properties of compounds identified in screens, generally referred to as lead compounds, are known in the art and comprise a method of modifying a compound identified as a lead compound to achieve: (i) modified site of action, spectrum of activity, organ specificity, and/or (ii) improved potency, and/or (iii) decreased toxicity (improved therapeutic index), and/or (iv) decreased side effects, and/or (v) modified onset of therapeutic action, duration of effect, and/or (vi) modified pharmacokinetic parameters (resorption, distribution, metabolism and excretion), and/or (vii) modified physico-chemical parameters (solubility, hygroscopicity, color, taste, odor, stability, state), and/or (viii) improved general specificity, organ/tissue specificity, and/or (ix) optimized application form and route by (i) esterification of carboxyl groups, or (ii) esterification of hydroxyl groups with carbon acids, or (iii) esterification of hydroxyl groups to, e.g. phosphates,

pyrophosphates or sulfates or hemi succinates, or (iv) formation of pharmaceutically acceptable salts, or (v) formation of pharmaceutically acceptable complexes, or (vi) synthesis of pharmacologically active polymers, or (vii) introduction of hydrophilic moieties, or (viii) introduction/exchange of substituents on aromates or side chains, change of substituent pattern, or (ix) modification by introduction of isosteric or bioisosteric moieties, or (x) synthesis of homologous compounds, or (xi) introduction of branched side chains, or (xii) conversion of alkyl substituents to cyclic analogues, or (xiii) derivatisation of hydroxyl group to ketales, acetales, or (xiv) N-acetylation to amides, phenylcarbamates, or (xv) synthesis of Mannich bases, imines, or (xvi) transformation of ketones or aldehydes to Schiff's bases, oximes, acetales, ketales, enolesters, oxazolidines, thiozolidines or combinations thereof; said method optionally further comprising the steps of the above described methods.

The various steps recited above are generally known in the art. They include or rely on quantitative structure-action relationship (QSAR) analyses (Kubinyi, "Hausch-Analysis and Related Approaches", VCH Verlag, Weinheim, 1992), combinatorial biochemistry, classical chemistry and others (see, for example, Holzgrabe and Bechtold, Deutsche Apotheker Zeitung 140(8), 813-823, 2000).

Clinical trials according to the invention can for example be performed as described in the following protocol.

Protocols for clinical trials

Response to treatment by an antidepressant, time until response (i.e. latency) to an antidepressant, or patient outcome to an antidepressant treatment protocol can be predicted by determining in patients the presence of at least one of the FKBP51 SNPs disclosed in Table 1. Accordingly, an individual homozygous for the TT allele of rs2766534, or the AA allele of rs4711429, or the TT allele of rs4713921, or the CC allele of rs9462104, or the GG allele of rs9394312, or the AA allele of rs4713916, or the AA allele of rs943297, or the GG allele of rs9380528, or the CC allele of rs9380526, or the GG allele of rs10947563, or the GG allele of rs9380525, or the AA allele of rs6912833, or the TT alllele of rs2143404, or the TT allele of rs1360780, or the GG allele of rs1591365, or the TT allele of rs7748266, or the GG allele of

rs9470069, or the CC allele of rs6926133, or the GG allele of rs3777747, or the GG allele of rs4713899 or the GG allele of rs2395634, or the GG allele of rs7753746, or the CC allele of rs3800373, or the CC allele of rs10807151, or the TT allele of rs3800374, or the AA allele of rs9348978, or the CC allele of rs11751447, or the AA allele of rs2395631, or with the CC, CG or GG allele of FKBP5UT5A, or the GG, GA or AA allele of FKBP5UT5C, or with elevated FKBP51 expression or protein levels would have an improved, or quicker response to an antidepressant drug. Patients should have at least one of these geneotypes or alleles, but may have two, three, or any combination or number of all 30 SNPs.

Individuals with either a GG or TG allele of rs2766534, a GG or AG allele of rs4711429, a CC or TC allele of rs4713921, a TT or CT allele of rs9462104, a CC or GC allele of rs9394312, or the a GG or AG allele of rs4713916, a GG or AG allele of rs943297, a AA or GA allele of rs9380528, a TT or CT allele of rs9380526, a AA or GA allele of rs10947563, a CC or GC allele of rs9380525, a TT or AT allele of rs6912833, a CC or TC alllele of rs2143404, a CC or CT allele of rs1360780, a AA or GA allele of rs1591365, a CC or TC allele of rs7748266, a CC or GC allele of rs9470069, a AA or CA allele of rs6926133, a AA or GA allele of rs3777747, a AA or GA allele of rs4713899, a AA or GA allele of rs2395634, a AA or GA allele of rs7753746, a AA or AC allele of rs3800373, aTT or CT allele of rs10807151, a CC or CT allele of rs3800374, a GG or AG allele of rs9348978, a TT or CT allele of rs11751447, a GG or AG allele of rs2395631, or low FKBP51 expression or low FKBP51 protein level would have an inferior, or slower response to an antidepressant drug. The various alleles are summarised in the following table.

Table 5

SNP	Column A Allele associated with improved response	Column B Alleles associated with inferior response
rs2766534	TT	GG or TG
rs4711429	AA	GG or AG
rs4713921	TT	CC or TC
rs9462104	CC	TT or CT
rs9394312	GG	CC or GC

rs4713916	AA	GG or AG
rs943297	AA	GG or AG
rs9380528	GG	AA or GA
FKBP5UT5A	CC or CG or GG	--
FKBP5UT5C	GG or GA or AA	--
rs9380526	CC	TT or CT
rs10947563	GG	AA or GA
rs9380525	GG	CC or GC
rs6912833	AA	TT or AT
rs2143404	TT	CC or TC
rs1360780	TT	CC or CT
rs1591365	GG	AA or GA
rs7748266	TT	CC or TC
rs9470069	GG	CC or GC
rs6926133	CC	AA or CA
rs3777747	GG	AA or GA
rs4713899	GG	AA or GA
rs2395634	GG	AA or GA
rs7753746	GG	AA or GA
rs3800373	CC	AA or AC
rs10807151	CC	TT or CT
rs3800374	TT	CC or TC
rs9348978	AA	GG or AG
rs11751447	CC	TT or CT
rs2395631	AA	GG or AG

In a clinical trial for a new antidepressant drugs, patients would be grouped or selected according to the presence of one or more of the alleles found in column A. In addition, patients could be selected according to elevated FKBP51 levels. Such patients would then be enrolled into a clinical trial for testing the efficacy of a new antidepressant drug where the endpoint would be improvement in the Hamilton Depression Rating score, dexamethasone suppression test, or combined dexamethasone - corticotropin releasing hormone test. Results obtained with this group of patients could be compared to a second group of patients which possess

one or more of the alleles found in column B or a group of patients with low FKBP51 expression.

In a more preferred embodiment, the method of classifying patients suffering from depression further comprises the step of treating said patient with an antidepressant.

The present invention also relates to a nucleic acid molecule comprising the sequence of SEQ ID NO: 116 or/and 118. This embodiment relates to sequences comprising the SNP(s) designated FKBP5UT5A or/and FKBP5UT5C and disclosed herein for the first time. All sequences comprising the FKBP51 locus or parts thereof and optionally adjacent regions are encompassed, provided they comprise the SNP(s) defined by SEQ ID NO: 116 or/and 118. The remainder of the comprised sequence from the FKBP51 locus and optionally adjacent regions may be wildtype sequence throughout, or may exhibit one or any combination of more than one of the polymorphisms disclosed herein and/or described in the art. It is understood that the wording "comprising the SNP(s) defined by SEQ ID NO: 116 or/and 118" as used herein refers to sequences comprising the polymorphic nucleotide site(s) in SEQ ID NO: 116 or/and 118. Such sequences may comprise SEQ ID NO: 116 or/and 118 in their entirety, but may also comprise only part thereof, with the proviso that the polymorphic site is present. Accordingly, also sequences beginning or ending with the polymorphic site in SEQ ID NO: 116 or 118 are included.

In view of the favourable response to therapy associated with elevated levels of FKBP51, the invention also provides a pharmaceutical composition comprising (a) a nucleic acid encoding a polypeptide with the sequence of SwissProt accession number Q13451; (b) a nucleic acid hybridizing to the complementary strand of the nucleic acid of (a) and encoding a polypeptide having the biological activity of the polypeptide with the sequence of SwissProt accession number Q13451; and/or (c) a polypeptide encoded by the nucleic acid of (a) or (b).

Said nucleic acids are provided in a formulation and to be administered via a route which ensures their expression.

Preferably, said hybridizing occurs under stringent conditions.

SwissProt (http://us.expasy.org/) is a manually curated database of annotated protein sequences. The recited identifier (Q13451) refers to the SwissProt entry for human FK506-binding protein 51 (FKBP51), sometimes also referred to as FKBP5, FKBP54 or AIG6. FKBP51 acts as a peptidyl-prolyl cis-trans isomerase (rotamase).

The invention also relates to the use of (a) a nucleic acid encoding a polypeptide with the sequence of SwissProt accession number Q13451; (b) a nucleic acid hybridizing to the complementary strand of the nucleic acid of (a) and encoding a polypeptide having the biological activity of the polypeptide with the sequence of SwissProt accession number Q13451; (c) a polypeptide encoded by the nucleic acid of (a) or (b); (d) an activator of the expression of the polypeptide with the sequence of SwissProt accession number Q13451; and/or (e) an activator of the polypeptide with the sequence of SwissProt accession number Q13451 for the manufacture of a pharmaceutical composition for the treatment of depression. Said activator of expression can be obtained by the above described screen for compounds that alter the promoter activity of FKBP51. Said activator of the polypeptide can be identified by the above described screen for compounds that bind FKBP51 and change its function or stability.

Furthermore, the invention provides a method of treating depression comprising the administration of (a) a nucleic acid encoding a polypeptide with the sequence of SwissProt accession number Q13451; (b) a nucleic acid hybridizing to the complementary strand of the nucleic acid of (a) and encoding a polypeptide having the biological activity of the polypeptide with the sequence of SwissProt accession number Q13451; (c) a polypeptide encoded by the nucleic acid of (a) or (b); (d) an activator of the expression of the polypeptide with the sequence of SwissProt accession number Q13451; and/or (e) an activator of the polypeptide with the sequence of SwissProt accession number Q13451 to a patient suffering from depression.

Recent studies have shown that FKBP51 and FKBP52 are physiologically relevant regulators of glucocorticoid receptor (GR) sensitivity. In the absence of steroids, FKBP51 interacts with the GR complex to retain it in the cytoplasm where it is

inactive. Upon steroid binding, FKBP51 is replaced by FKBP52 which results in a recruitment of dynein into the GR complex, allowing nuclear translocation of the complex and transcriptional activity of the glucocorticoid receptor (Davies et al., J. Biol. Chem. 227 (2002), 4597-4600).

Thus, inhibitors of FKBP52 may have antidepressant effects by allowing FKBP51 to remain in contact with the GR complex and keeping the glucocorticoid receptor in an cytoplasmic inactive form despite steroid binding. The term "inhibitor of FKBP52" refers to substances inhibiting the biochemical or catalytic activity and/or the biological activity of FKBP52. The latter class of substances comprises compounds interfering with the interaction between FKBP52 and its interaction partners. Said interaction partners comprise components of the GR complex, including heat shock proteins such as Hsp90.

There are several examples of known or recognised FKBP52 inhibitors described in the art. Furthermore, inhibitors of immunophilins (or pepidyl-prolyl cis-transisomerases or rotamases) in general or of any specific immunophilin other than FKBP52 is a potential inhibitor of FKBP52. Whether such substance does inhibit FKBP52 can be assayed with the screens or assays described above.

A screening method to identify inhibitors of FKBP52 binding to partner proteins such as Hsp90 can be performed using bioluminescence resonance energy transfer (BRET). Briefly, the FKBP52 protein is fused to Renilla luciferase, whereas the Hsp90 protein is fused to a fluorescent protein, for example the yellow fluorescent protein (YFP). The emission of the luciferase signal is detected following addition of its substrate coelenterazine. When FKBP52 binds to Hsp90, a resonance energy transfer occurs between the luciferase and the YFP resulting in the detection of both the luciferase and the YFP signals. An inhibitor compound preventing binding of FKBP52 to Hsp90 would result in the emission of the luciferase signal only. The use of BRET for drug screening is well known in the art, see for example Xu, Y. et al. (1999) A bioluminescence resonance energy transfer (BRET) system: application to interacting circadian clock proteins. Proc. Natl. Acad. Sci. U. S. A. 96: 151-156; Boute et al. (2002) The use of resonance energy transfer in high-throughput screening: BRET versus FRET. Trends Pharmacol. Sci. 23:351-354.

Identification of inhibitors of FKBP52 protein binding can also be done using the CheckMate™ Mammalian Two-Hybrid System from Promega or fluorescence resonance energy transfer (FRET). Such methods are well known in the art, see for example Serebriiskii et al. (2002) Detection of peptides, proteins, and drugs that selectively interact with protein targets. Genome Res. 12:1785-1791; Bergendahl et al. (2003) Luminescence resonance energy transfer-based high-throughput screening assay for inhibitors of essential protein-protein interactions in bacterial RNA polymerase. Appl. Environ. Microbiol. 69:1492-1498.

International Patent Applications WO 98/13343, WO 98/13355, WO 98/20891, WO 98/20892 and WO 98/20893 describe various pyrrolidine, piperidine and homopiperidine derivatives having an acyl, amide, oxalyl, or similar linking group, at the 1-position of the heterocycle.

US 5,721,256 describes various pyrrolidine, piperidine and homopiperidine derivatives having an SO_2 linking group at the 1-position of the heterocycle.

US 6,166,011 and US 6,509,464 disclose compounds that inhibit FKBP12 and FKBP52 without inhibiting the protein phosphatase calcineurin and therefore lack any significant immunosuppressive activity.

WO 99/21552 discloses an FKBP52 antibody and geldanamycin derivatives that can disrupt and inhibit the association between FKBP52 and Hsp90.

The present invention provides a pharmaceutical composition containing a recognised inhibitor of FKBP52.

The present invention also relates to the use of (a) geldanamycin or a geldanamycin derivative that can disrupt and inhibit the association between FKBP52 and Hsp90; and/or (b) an antibody or an aptamer specifically recognizing FKBP52 or a fragment or epitope thereof for the manufacture of a pharmaceutical composition for the treatment of depression. Suitable geldanamycin derivatives or analogs can be identified by the screens described above.

In a preferred embodiment, said inhibitor is an antibody or an aptamer specifically recognizing FKBP52 or a fragment or epitope thereof. Said antibody may be a

monoclonal or a polyclonal antibody.

A more preferred embodiment of the invention relates to an antibody which is a monoclonal antibody.

Said antibody, which is monoclonal antibody, polyclonal antibody, single chain antibody, or fragment thereof that specifically binds FKBP52 also includes a bispecific antibody, synthetic antibody, antibody fragment, such as Fab, a $F(ab_2)'$, Fv or scFv fragments etc., or a chemically modified derivative of any of these (all comprised by the term "antibody"). Monoclonal antibodies can be prepared, for example, by the techniques as originally described in Köhler and Milstein, Nature 256 (1975), 495, and Galfré, Meth. Enzymol. 73 (1981), 3, which comprise the fusion of mouse myeloma cells to spleen cells derived from immunized mammals with modifications developed by the art. Furthermore, antibodies or fragments thereof to the FKBP52 can be obtained by using methods which are described, e.g., in Harlow and Lane "Antibodies, A Laboratory Manual", CSH Press, Cold Spring Harbor, 1988. When derivatives of said antibodies are obtained by the phage display technique, surface plasmon resonance as employed in the BIAcore system can be used to increase the efficiency of phage antibodies which bind to an epitope of FKBP52 (Schier, Human Antibodies Hybridomas 7 (1996), 97-105; Malmborg, J. Immunol. Methods 183 (1995), 7-13). The production of chimeric antibodies is described, for example, in WO89/09622. A further source of antibodies to be utilized in accordance with the present invention are so-called xenogenic antibodies. The general principle for the production of xenogenic antibodies such as human antibodies in mice is described in, e.g., WO 91/10741, WO 94/02602, WO 96/34096 and WO 96/33735. Antibodies to be employed in accordance with the invention or their corresponding immunoglobulin chain(s) can be further modified using conventional techniques known in the art, for example, by using amino acid deletion(s), insertion(s), substitution(s), addition(s), and/or recombination(s) and/or any other modification(s) known in the art either alone or in combination. Methods for introducing such modifications in the DNA sequence underlying the amino acid sequence of an immunoglobulin chain are well known to the person skilled in the art; see, e.g., Sambrook (1989), loc. cit..

The term "monoclonal" or "polyclonal antibody" (see Harlow and Lane, (1988), loc. cit.) also relates to derivatives of said antibodies which retain or essentially retain their binding specificity. Whereas particularly preferred embodiments of said derivatives are specified further herein below, other preferred derivatives of such antibodies are chimeric antibodies comprising, for example, a mouse or rat variable region and a human constant region.

The term "scFv fragment" (single-chain Fv fragment) is well understood in the art and preferred due to its small size and the possibility to recombinantly produce such fragments.

The term "specifically binds" in connection with the antibody used in accordance with the present invention means that the antibody etc. does not or essentially does not cross-react with (poly)peptides of similar structures. Cross-reactivity of a panel of antibodies etc. under investigation may be tested, for example, by assessing binding of said panel of antibodies etc. under conventional conditions (see, e.g., Harlow and Lane, (1988), loc. cit.) to the (poly)peptide of interest as well as to a number of more or less (structurally and/or functionally) closely related (poly)peptides. Only those antibodies that bind to the (poly)peptide/protein of interest but do not or do not essentially bind to any of the other (poly)peptides which are preferably expressed by the same tissue as the (poly)peptide of interest, are considered specific for the (poly)peptide/protein of interest and selected for further studies in accordance with the invention.

The invention also provides a method of treating depression comprising the administration of (a) geldanamycin or a geldanamycin derivative that can disrupt and inhibit the association between FKBP52 and Hsp90; and/or (b) an antibody or an aptamer specifically recognizing FKBP52 or a fragment or epitope thereof to a patient suffering from depression.

The Figures show:

Figure 1. Representation of linkage disequilibrium (LD) structure using D' in the FKBP51 region. The 4 SNPs of the invention are labelled with a red arrow. The quantity D' (D prime) measures the amount of linkage disequilibrium between alleles at two loci. It is defined as the difference between an observed haplotype frequency and its expected value under the assumption of no disequilibrium. This difference is then divided by its theoretical maximum. The range of D' therefore goes from 0, indicating no linkage disequilibrium to 1, indicating maximum disequilibrium. D' is defined and first published by Lewontin in 1964[27].

Figure 2. Association of SNPs in the FKBP51 gene region and response to antidepressant drugs at week 2. The –log p values (y axis) of the association were plotted against the physical location of the SNPs (x axis) on chromosome 6 according to version hg15 of the UCSC genome draft.

Figure 3a. Hamilton Depression Rating Scale (HAM-D) scores over the first 5 weeks of hospitalisation according to the rs1360780 genotype with all patients. A repeated measures ANOVA showed an overall significant genotype effect: $F_{2,197}$= 5.3, p = 0.0058. Patients did not differ in disease severity at admission: mean HAM-D score at admission (SD) for CC = 26.2 (7.3); CT = 25.7 (8.1); TT = 26.2 (7.2). A one way ANOVA showed a non-significant genotype effect for differences in HAM-D scores at admission: $F_{2,277}$ = 0.15, p = 0.86.

Figure 3b. Hamilton Depression Rating Scale (HAM-D) scores over the first 5 weeks of hospitalisation according to the rs1360780 genotype with patients treated with selective serotonin reuptake inhibitors (N = 70). A repeated measures ANOVA shows a significant interaction of rs1360780 genotype and change in HAM-D scores, $F_{4.3,170.5\ (Greenhouse\text{-}Geisser)}$ = 2.67; p = 0.03.

Figure 3c. Hamilton Depression Rating Scale (HAM-D) scores over the first 5 weeks of hospitalisation according to the rs1360780 genotype with patients treated with tricyclic antidepressants (N = 48). A repeated measures ANOVA shows a significant effect of rs1360780 genotype on change in HAM-D scores, $F_{2,45}$ = 3.43; p = 0.04.

Figure 3d. Hamilton Depression Rating Scale (HAM-D) scores over the first 5 weeks of hospitalisation according to the rs1360780 genotype with patients treated with mirtazapine (N = 55). A repeated measures ANOVA shows a significant effect of rs1360780 genotype on change in HAM-D scores, $F_{2,52} = 4.2$; $p = 0.02$.

Figure 4. Effect of rs1360780 genotype on ACTH response in the Dex-CRH test at admission and discharge for all patients. A t-test showed that peak ACTH values (T = 3.48; df = 95.8; p = 0.00075) and area under the curve of the ACTH response (T = 1.92; df = 98.1; p = 0.056) were significantly lower in the TT genotype vs. the two other genotypes. The cortisol response at admission and the ACTH and cortisol responses at discharge were not significantly different between these two groups.

Figure 5. Number of previous depressive episodes and rs1360780 genotype for all patients.

Figure 6a. HAM-D scores over the first 5 weeks of hospitalisation against rs4713916 genotypes. A repeated measures ANOVA showed an overall significant genotype effect: F2,125 = 4.75, p = 0.012 and interaction between response and genotype: F4.761,511.812 = 2.6, p = 0.03. Patients did not differ in disease severity at admission: mean HAM-D score at admission (SD) for AA = 26.3 (7.0); AG = 27.4 (8.3); GG = 26.8 (7.6). A one-way ANOVA showed a non-significant genotype effect for differences in HAM-D scores at admission: F2,436, = 0.40, p = 0.67.

Figure 6b. Effect of rs4713916 genotype on ACTH response in the Dex-CRH test at admission and discharge for all patients. Results show a tendency for lower ACTH response in the AA genotype vs. the two other genotypes.

Figure 6c. Number of previous depressive episodes and rs4713916 genotype for all patients.

Figure 6d. Duration of treatment with antidepressants until response for each rs4713916 genotype for all patients.

Figure 7a. HAM-D scores over the first 5 weeks of hospitalisation against rs3800373 genotypes. A repeated measures ANOVA showed an overall significant genotype effect: $F_{2,214}$= 7.76, $p < 0.001$ and interaction between response and genotype: 2,214 = 4.5, $p = 0.012$. Patients did not differ in disease severity at admission: mean HAM-D score at admission (SD) for CC = 25.6 (6.9); CA = 26.0 (8.2); AA = 27.9 (7.3). A one-way ANOVA showed a non-significant genotype effect for differences in HAM-D scores at admission: $F_{2,435} = 0.38$, $p = 0.686$.

Figure 7b. Effect of rs3800373 genotype on ACTH response in the Dex-CRH test at admission and discharge for all patients. A t-test showed that the area under the curve of the ACTH response ($T = 2.273$; $df = 38.746$; $p = 0.029$) was significantly lower in the AA genotype vs. the two other genotypes. Peak ACTH values were also lower in the AA genotype than the two other genotypes ($p < 0.07$). The cortisol response at admission and the ACTH and cortisol responses at discharge were not significantly different between these two groups.

Figure 7c. Number of previous depressive episodes and rs3800373 genotype for all patients.

Figure 7d. Duration of treatment with antidepressants until response for each rs3800373 genotype for all patients.

Figure 8. Western blot of FKBP51 protein for the various genotype of the rs1360780 SNP.

Figure 9. FKBP51 protein levels for the various genotype of the rs1360780 SNP. Quantitative difference in FKBP51 protein expression levels in lymphocytes according to rs1360780 genotypes (mean ± SEM). The data represents the average values of two independent replications of the western blot analysis. A one-way ANOVA with patient status and plasma cortisol levels as covariates showed a significant genotype effect (TT vs. two other genotypes) for differences in FKBP51 levels: $F_{1,25} = 5.96$; $p = 0.024$. Patient status ($F_{1,25} = 0.35$; $p = 0.55$) and plasma cortisol levels ($F_{1,25} = 0.01$; $p = 0.95$) had no significant influence on FKBP51 levels,

Controls: N = 18, patients: N = 7; TT: N = 12, CT: N = 5, CC: N = 8. rec = recombinant FKBP51.

Figure 10. Quantitative difference in FKBP51 mRNA expression in peripheral blood monocytes of healthy controls according to the rs1360780 genotypes (mean ± SEM). A one way ANOVA revealed no significant genotype effect (N = 124).

Figure 11. A. Correlation of plasma cortisol levels (X axis) and relative FKBP51 mRNA levels in PBMCs in healthy controls, all rs1360780 genotypes combined (N = 124). **B.** Correlation of plasma cortisol levels (X axis) and relative FKBP51 mRNA levels in PBMCs in healthy controls with CC and TC as rs1360780 genotypes (N = 103). **C.** Correlation of plasma cortisol levels (X axis) and relative FKBP51 mRNA levels in PBMCs in healthy controls with TT as rs1360780 genotype (N = 21). For parts A-C ** = p values for correlation < 0.01.

The Examples illustrate the invention.

Example 1

Methods

Patients:

Between 256 and 294 patients admitted to our psychiatric hospital for treatment of a depressive disorder presenting with a unipolar depressive episode (86.6%), bipolar disorder (12.0%, 7.5% bipolar I and 4.5% bipolar II) or dysthymia (1.2%) as their primary psychiatric diagnoses were recruited for the study. For 30.8% these patients the depressive episode could be specified as with melancholic features. Patients were included in the study within 1-3 days of admission to our hospital and the diagnosis was ascertained by trained psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders (DSM) IV criteria. Depressive disorders due to a medical or neurological condition were exclusion criteria. Ethnicity was recorded using a self-report sheet for perceived nationality, mother language and ethnicity of the subject itself and all 4 grandparents. All included patients were Caucasian and 92 % of German origin. An additional 50 patients were genotyped for the investigation of quantitative phenotypes. The study has been approved by the local ethics committee. Written informed consent was obtained from all subjects. Severity of psychopathology at admission was assessed using the 21 items Hamilton Depression Rating Scale (HAM-D) by trained raters, including residents in psychiatry and psychologists. Ratings were performed within 3 days of admission and then in weekly intervals until discharge. We used three types of response definition commonly used in psychiatric research, that define different aspects of antidepressant response. Early response at two weeks was defined as a greater than 25% decrease of HAM-D scores from the score obtained at admission. Patients with a reduction > 25% from their score at admission were considered as early responders, while patients whose HAM-D score decreased ≤ 25% from the score at admission were considered as early non-responders. For the definition of response at 5 weeks a reduction of over 50% in HAM-D scores at admission was required to be considered a responder for these time points. All patients with a reduction of HAM-D scores equal to or less than 50% were assigned to the group of non-

responders. The five-week time point was chosen because this duration of treatment is considered sufficient for an antidepressant drug to display its clinical efficacy. Remission at discharge was defined as reaching a total HAM-D score equal or smaller than 10. Overall response at discharge may serve to investigate aspects of treatment resistance, which can occur in up to 10-15% of all patients. The study was designed as a naturalistic pharmacogenetic study (Munich Antidepressant Response Signature (MARS) project (Holsboer 2001)), all patients were treated according to the doctor's choice with antidepressant drugs within a few days of admission. Patients who were admitted on antidepressant medication (73%) were switched to another compound within a few days of admission. 21.6 % of patients received a mood stabilizer in addition to the antidepressant medication and 7.7% an antipsychotic drug. 48.7 % of patients were additionally treated with a benzodiazepine in the first few days after admission. For all patients plasma concentration of antidepressant medication was monitored to assure clinically efficient drug levels. Not all patients finished the weekly psychopathology ratings after being included in the study.

Patients for the replication sample (N = 85) were recruited at the psychiatric hospital of the Ludwig Maximilian University in Munich, Germany (N = 57) and the psychiatric hospitals of Augsburg (N = 12) and Ingolstadt (N = 16), Germany. 89.8% had a unipolar affective disorder, 8.2 % a bipolar affective disorder (5.9% bipolar I and 2.3% bipolar II) and 2.0% dysthymia. 66.0% were female and 34.0% were male. The mean age was 50.5 years (SD:12.48). Patients from these hospitals were recruited for pharmacogenetic studies. Patients from the Ludwig Maximilian University were treated in monotherapy studies for 4 weeks, 18 patients with sertraline, 15 with reboxetine and 24 with mirtazapine. In Ingolstadt and Augsburg the studies also have a naturalistic design. The two most frequently used primary antidepressants were mirtazapine (45.9%) and citalopram (27.3%). In all three hospitals, diagnosis was assertained by trained psychiatrist according to DSMIV and patients were rated weekly from admission to discharge using the HAM-D rating scale. At the Ludwig Maximilian University patients were also rated using the Clinical Global Impression Scale (CGI) and the Montgomery Asperg Depression Rating Scale (MADRS). For the purpose of this study early response at two weeks was defined using the same criteria as for the Max-Planck Institute of Psychiatry sample (patients with a reduction > 25% from their score at admission were considered as early responders, while patients whose HAM-D score decreased ≤ 25% from the score at admission were

considered as early non-responders).The studies had also been approved by the local ethics committee. Written informed consent was obtained from all subjects.

339 controls matched for ethnicity (using the same questionnaire as for patients), sex: 41.4% male in patients and 37.0% male in controls (p = 0.24) and age (mean age of 47.65, SD 14.5 in patients and mean age of 46.59, SD 15.4 in controls, p = 0.363) were recruited. Controls were selected randomly from a Munich-based community sample and screened for the presence of anxiety and affective disorders using the Composite International Diagnostic-Screener (Wittchen, H. et al. Screening for mental disorders: performance of the Composite International Diagnostic - Screener (CID-S). International Journal of Methods in Psychiatric Research 8, 59-70). Only individuals negative for the above-named disorders were included in the sample. Recruitment of controls was also approved by the local ethics committee and written informed consent was obtained from all subjects.

DNA preparation:

On enrollment in the study, 40 ml of EDTA blood were drawn from each patient and DNA was extracted from fresh blood using the Puregene® whole blood DNA-extraction kit (Gentra Systems Inc; MN).

Genotyping:

Up to 1000 bp of the promoter region and all exons including at least 50 bp of the exon/intron junctions were screened in 94 depressed patients, for potential genetic differences in all investigated genes except GR were screened using capillary electrophoresis single strand conformational polymorphism (CE-SSCP) analysis in 94 depressed patients. Subsequent sequencing of PCR products showing mobility differences revealed 9 SNPs with minor allele frequencies ranging from 0.022 to 0.364. Except for one intronic polymorphism in STUB1 (rs1046112) none of the other polymorphisms had been recorded in dbSNP as of March 2003 (see supplemental information on http//www.mpipsykl.mpg.de/AGBMM for sequences). CE-SSCP and subsequent sequencing were performed on an ABI PRISM® 3100 DNA sequencer (Applied Biosystems; CA) using standard procedures. SNPs in the FKBP51 locus were selected from the SNPs identified by (CE-SSCP) analysis screening in 94 patients, from the public SNP database dbSNP (http://www.ncbi.nlm.nih.gov:80/). The SNP search tool at http://ihg.gsf.de/ihg/snps.html was used to download SNP

sequences from public databases. All newly detected SNPs were selected for genotyping and supplemented by SNPs from dbSNP. Prior to genotyping, the relevant regions were amplified by PCR. Table 6 shows the PCR primers used. Genotyping was performed on a MALDI-TOF mass-spectrometer (MassArray® system) employing the Spectrodesigner software (Sequenom™; CA) for primer selection and multiplexing and the homogeneous mass-extension (hME) process for producing primer extension products. Table 7 shows the primers used for the primer extension assays.

Table 6

SNP-ID	sense	antisense
rs1334894	ACGTTGGATGCTTGTCATCCCAGCATCTTC	ACGTTGGATGCCCTATTTGGCCTCAAAAAG
rs1360780	ACGTTGGATGAAGAGATCCAGGCACAGAAG	ACGTTGGATGTGCCAGCAGTAGCAAGTAAG
rs15258	ACGTTGGATGGTCAGTATGGCAAATGAGTG	ACGTTGGATGTTTTCAGCCCGGGTATAAGC
rs1546428	ACGTTGGATGAAACGGAAGCAGAGTCACTC	ACGTTGGATGGGCTGAAAATCCGGAAAAAG
rs1802039	ACGTTGGATGGAGGTGTAGCAGAAAAAGGC	ACGTTGGATGTCAGGTAAATTCCGCTCCAG
rs1803817	ACGTTGGATGTGGAGAGGGGCTATGCTTCTG	ACGTTGGATGCACAGAGATGAAGGAGGAGC
rs1903370	ACGTTGGATGCATTGTGTATCCTTCCAGGC	ACGTTGGATGAGGGACTACAAATCAGTCTG
rs1981656	ACGTTGGATGTACTCACTTTCTCCCCCTTC	ACGTTGGATGTAGTGGACAAAGACTCGGTC
rs2118727	ACGTTGGATGAGCTCCAAAGTATGCTACCG	ACGTTGGATGTCTAGCCCACAGTTGTGATG
rs2290893	ACGTTGGATGAGCCTTCTCCAAGATAGGTC	ACGTTGGATGGCTCTTCAGCGATTTCGTAG
rs258813	ACGTTGGATGTTGATCATGGGAAGACATGG	ACGTTGGATGTAGGATGCGCCTTTTTCTCC
rs2740204	ACGTTGGATGTGTGTTTCTCTGGGTGTCTG	ACGTTGGATGAACTGCTAAGAACGTGTCCG
rs2958153	ACGTTGGATGAAGGCCTAGGGTGAAGTTAC	ACGTTGGATGCCTACTTTTACCCCTTTGGC
rs2958154	ACGTTGGATGCCAGCATCACTACATGA	ACGTTGGATGGCATGTTTCAGAGTCCA

	TAG	GAG
rs3021522	ACGTTGGATGACCCCAGGAGAGAAACA AAG	ACGTTGGATGCAGGGTCTTCCCATCTA AAG
rs3176921	ACGTTGGATGTATGAGCCTGCAGAAGC AAG	ACGTTGGATGTGGGAGTAGCTCTTGTC ATC
rs3204090	ACGTTGGATGTCCCCAACTTGGCTATG AAG	ACGTTGGATGAGGGAACCTCAGTAGTC CTC
rs33388	ACGTTGGATGTCTGCCTGAATGAATGG GTG	ACGTTGGATGTGGGTGAAAGTCATGGA TGG
rs33391	ACGTTGGATGCACTACAGGAGTCTCAC AAG	ACGTTGGATGGGTGAGTTGTGGTAACG TTG
rs5195	ACGTTGGATGTGCGCTGCCAGGAGGA GAAC	ACGTTGGATGTTGCAGCAAACGCCGAA GGC
rs6156	ACGTTGGATGGGGAAAGAGAGGAGAG AAAG	ACGTTGGATGTCTCTGCAGAGAGACGT CTC
rs6157	ACGTTGGATGAAAGTTGGTGGCGTGTT CCG	ACGTTGGATGAAGGAAGACAACCTCCA GAG
rs6158	ACGTTGGATGAAAGTTGGTGGCGTGTT CCG	ACGTTGGATGAGACGTCTCTCTGCAGA GAG
rs6159	ACGTTGGATGTGGGAGAGGAGTACTTC CTC	ACGTTGGATGAAAAAGTTGGCGGTCGC CTG
rs6188	ACGTTGGATGTAAACTGTGTAGCGCAG ACC	ACGTTGGATGTGTAGTGGCCTGCTGAA TTC
rs6191	ACGTTGGATGTTTCCATCTTGGCTGGT CAC	ACGTTGGATGCCTTCTGACACTAAAAC CAG
rs6192	ACGTTGGATGCCAAGCAGCGAAGACTT TTG	ACGTTGGATGACTGCTTTGGACAGATC TGG
rs6195	ACGTTGGATGCATTCCACCAATTCCCG TTG	ACGTTGGATGTCCCCAGAGAAGTCAAG TTG
rs6196	ACGTTGGATGGGCAGTCACTTTTGATG AAAC	ACGTTGGATGCCGAGATGTTAGCTGAA ATC
rs6597	ACGTTGGATGCTGGCAAGCAGGAAATG TGG	ACGTTGGATGAGATGGACCTGGCCAGA TGTC
rs706118	ACGTTGGATGAATGTCTGGGCCATAGG AAC	ACGTTGGATGTTTCCTGTCACAATGAG CCC
rs706120	ACGTTGGATGAGACTGGAACTCAGGAC TTG	ACGTTGGATGTAGGTCTAGCAGCTTTC TGC
rs734369	ACGTTGGATGATAAGGCTGCAAGACTG CAG	ACGTTGGATGATGTTGGCGTATATCCT GCG
rs737054	ACGTTGGATGACAAGGTGACCGAGAAC ATG	ACGTTGGATGGGTATATTTGGTCAGGT GCC

rs755658	ACGTTGGATGACTAGGATTTACCACAG CCC	ACGTTGGATGGGTGAGGGTTGGAGTAT TTG
rs2103681	ACGTTGGATGCTTGAGCTGACATGAGC ATC	ACGTTGGATGAACAGCTGACCCACAGA ATC
rs1883636	ACGTTGGATGAGTCTTGAGGGTTCACT CAG	ACGTTGGATGTCTTACTGGGCCCAAAC AAG
rs1540910	ACGTTGGATGTGCTCCAAAGTCCCTAT TCC	ACGTTGGATGTTGCATGTCTGGAGATC TGG
rs4713878	ACGTTGGATGCTAAACACTGGCTGTGT GAC	ACGTTGGATGTTCATCCTCACAGCACA CTC
rs1883637	ACGTTGGATGAGAAGCTGGGCAGATTT CTC	ACGTTGGATGGGCCCTGAATCAGTCTT AAG
rs992105	ACGTTGGATGGGCATGGCCTTAACTTT GTG	ACGTTGGATGGAACGTACTCTGGTAAG CAC
rs1051952	ACGTTGGATGAACATCTGGAGTTGGAG CCG	ACGTTGGATGGGGAGAGGGGAGGTTA AA
rs2273000	ACGTTGGATGTAGAATCCTCTGTCCTC CTC	ACGTTGGATGAATTTCTCCCCACGATG GTC
rs4713897	ACGTTGGATGGCCCTGAGTATACTTTC TGG	ACGTTGGATGCACTGGGCATTTGCCCA TTT
rs734369	ACGTTGGATGATAAGGCTGCAAGACTG CAG	ACGTTGGATGATGTTGGCGTATATCCT GCG
rs747411	ACGTTGGATGGACGTGACACCACACTT GAC	ACGTTGGATGAGGCAGGAGGATCTCTT GAG
rs1320991	ACGTTGGATGGTGTGCGTATGCATACT GTG	ACGTTGGATGTGCCTGCACTGAAAACA TGC
rs737054	ACGTTGGATGACAAGGTGACCGAGAAC ATG	ACGTTGGATGGGTATATTTGGTCAGGT GCC
rs3777747	ACGTTGGATGCCACTCTTACATTCCTCT CC	ACGTTGGATGTCCCTCTCCAAATCTCA CTG
rs4401662	ACGTTGGATGAATTGCTTGAACCCAGG AGG	ACGTTGGATGAGTCTTGCTCTGTCATC CAG
rs4713908	ACGTTGGATGATTCCGTTGTGTGTGTAT GC	ACGTTGGATGTCAGACATCTGTCATTCT TC
rs755658	ACGTTGGATGACTAGGATTTACCACAG CCC	ACGTTGGATGGGTGAGGGTTGGAGTAT TTG
rs4713899	ACGTTGGATGAGTGGAGCTATAGGAGC TAG	ACGTTGGATGACGGAAAGACTGCTGAT TGC
rs1360780	ACGTTGGATGAAGAGATCCAGGCACAG AAG	ACGTTGGATGTGCCAGCAGTAGCAAGT AAG
rs4713907	ACGTTGGATGTCCTGACTTTGTGATCC	ACGTTGGATGATACCATACTCTAGGCT

	ACC	GGG
rs2092427	ACGTTGGATGACCTGCAGTACTTTTGG CAG	ACGTTGGATGCAGACACTTTCTAAGTG CTG
rs4713905	ACGTTGGATGTTCAATACCTCACCTGTC TC	ACGTTGGATGGTGTATTCTGCTTGTATT GGG
rs4520009	ACGTTGGATGACGAACAGGAAACTGAA AGC	ACGTTGGATGGAGCATGGTTTTCAGTA AAG
rs4713906	ACGTTGGATGAAGTATTTGTGGCTGGA GGC	ACGTTGGATGATCTCCTGACCTTGTGA TCC
rs4713916	ACGTTGGATGTATCTGGCAACCCTAAC CTC	ACGTTGGATGCCTAACGAGATAGTGAG GAG
rs2143404	ACGTTGGATGGGTTAGGTAGAGCTCAG TTC	ACGTTGGATGGTAGAGAACCTGGTAAG AAG
rs4711429	ACGTTGGATGACACACGGCTCATCTGT AAC	ACGTTGGATGATGCCAGGCATTTGGGT TTC
rs9462104	ACGTTGGATGTTTCCTCCCTAGATTCCC AG	ACGTTGGATGGTCCCAGTGGAACTTTA TGG
rs9394312	ACGTTGGATGACAAGCTATCGGGCTTT CTC	ACGTTGGATGAGACAATCTCCCAGTTG CAG
rs943297	ACGTTGGATGTCTGAAATCCAGCTGGA CAC	ACGTTGGATGAGTGAGACTCTGTCCAA AAG
rs9380528	ACGTTGGATGTTGAGACCAGATGTTGG AGG	ACGTTGGATGGACAGAGTCTGGCTCTG TTG
FKBP5UT5A	ACGTTGGATGTACAGACCCTGCAAAGA ACC	ACGTTGGATGTGCGCTATGGCTGCAGA TAC
FKBP5UT5C	ACGTTGGATGTACAGACCCTGCAAAGA ACC	ACGTTGGATGTGCGCTATGGCTGCAGA TAC
rs9380526	ACGTTGGATGAAACAGGGAAGCTTCTA GGC	ACGTTGGATGAAGCCAAGACTGAAACC GCC
rs10947563	ACGTTGGATGTATTACAGGCGTGAGCC ATC	ACGTTGGATGATGGTCTTTAGAGGAAT TCT
rs9380525	ACGTTGGATGGACAGAGTGAGACTCCT CTA	ACGTTGGATGTTCTCAAACCAGCACTC AGG
rs6912833	ACGTTGGATGGGGAAAAGAATCAAGGG AAG	ACGTTGGATGCTGGAACTAAGTGATCT GTG
rs7748266	ACGTTGGATGAGACCACACCACAGAAC AAG	ACGTTGGATGTTGCTTAACCCTCTCCA GAC
rs9470069	ACGTTGGATGATATACATACAGGCTGG CCG	ACGTTGGATGACTCCTGACCTCGTGAT CTG
rs6926133	ACGTTGGATGGATGACTGATACTTCAG TCT	ACGTTGGATGGAAAGCAAAGCTGAAAA GTAG

rs2395634	ACGTTGGATGTCGAAGGGACTTATTCCTCC	ACGTTGGATGCAGCAGAAGGAAGACATCAG
rs7753746	ACGTTGGATGAGAATAAACCTGTGTTTCTG	ACGTTGGATGAGGAGCAACCATATTTCTAA
rs10807151	ACGTTGGATGGGGTGAAAGTGGCAGAACAA	ACGTTGGATGCCGCTGGCTAATGAAAAAAC
rs9348978	ACGTTGGATGAATTTTTACCACTGAGCAGG	ACGTTGGATGGCTTTGGCTTTACGGAAGAG
rs11751447	ACGTTGGATGATTCTCTCTTGAGTCGGTGC	ACGTTGGATGTAGAACCTTGCCACAGAGAC
rs2395631	ACGTTGGATGTGCAAAAACTAACAAAAGCC	ACGTTGGATGAGGGTTATCAACCTTGAGGC
rs4713902	ACGTTGGATGGGAGCCAAAACATGAAGAGC	ACGTTGGATGTAGGCAACCTGTATAAGCTG
rs1334894	ACGTTGGATGCTTGTCATCCCAGCATCTTC	ACGTTGGATGCCCTATTTGGCCTCAAAAAG
rs1475774	ACGTTGGATGCAAGTGAAAAACTCCACACC	ACGTTGGATGCCATAAGTCTTTGTCCACAAG
rs2817035	ACGTTGGATGCCTCTTTTCTCCTAGGATCC	ACGTTGGATGGTTGCAAACAGAGGTAGGAG
rs3807050	ACGTTGGATGAGAGGGAGGGAATAGTTCAG	ACGTTGGATGTGTGTCTCCAAGACTGTGTG
rs1977655	ACGTTGGATGCCCGTCTCTGCTAAAAATAC	ACGTTGGATGCCAAGTTCAAGCGATTCTTG
rs3800374	ACGTTGGATGAGACGATGGACCCATTTTAC	ACGTTGGATGTCTTTTCCAAGTGGTGAACC
rs4713912	ACGTTGGATGAGGTTCAAGCGATTCTCCTG	ACGTTGGATGCAAAAATTAGCCGGGCTTGG
rs2817047	ACGTTGGATGTCTGGGCTCAAGTGATTCTC	ACGTTGGATGAATTAGCCAGGCATGATGGC
rs4713913	ACGTTGGATGGAATTTAACTAGGAGTGCTG	ACGTTGGATGAGCGAGACTCCGTCTCAAAA
rs4713921	ACGTTGGATGAAGCCCTGTGGTTTTATGCC	ACGTTGGATGTGGAACAATTCTGTCCCCAG
rs2817041	ACGTTGGATGGTACACACAGCGAGTGATAC	ACGTTGGATGCTCCTCAACTCTTTGGAGTG
rs2766543	ACGTTGGATGTCTGTGGTTGGCCTTTTCTC	ACGTTGGATGAAGCAGAGCTGCCCAATAAG
rs5020575	ACGTTGGATGTGATCTTGGCTCACTGCAAC	ACGTTGGATGAAATTAGCCAAGCGTGGTGG
rs1591365	ACGTTGGATGGTGGCAAATAGGAGTTC	ACGTTGGATGTTGGCAGGTGTTTTTCT

	TCC	GAG
rs2296662	ACGTTGGATGAATTGGCCTATGACCAG CAC	ACGTTGGATGAAGAGGAGGAGAAACCA GAG
rs2817010	ACGTTGGATGTGGTGTGTATGAGAAGC AGC	ACGTTGGATGTAAGTCTGTGCAGACGG TGG
rs873941	ACGTTGGATGGGGTGATGCTTTTGCAA CTC	ACGTTGGATGGCTATGATACCTGGCTG ATC
rs3800373	ACGTTGGATGAAACCCCTAGTGTAGAA GAG	ACGTTGGATGTTTACACTCCTCTATCAT GC
rs4713903	ACGTTGGATGGAGTCTAAACAAAAACA TCC	ACGTTGGATGAAGTACTGGGATTACAG GTG
rs2766534	ACGTTGGATGTCGAAGGGACTTATTCC TCC	ACGTTGGATGCAGCAGAAGGAAGACAT CAG
rs2766554	ACGTTGGATGAGAACCTCCTACTACTG AGC	ACGTTGGATGGCTTAGAATCCATGACC CAC
rs2817054	ACGTTGGATGAAGCTAAGGCCCAGGAC GTG	ACGTTGGATGACCTGTCAGCCCTCTGA GCT
rs2766597	ACGTTGGATGTTATAGCCCCATCACCA CAG	ACGTTGGATGAGGTTGATAATGATCCC CCG

Table 7

SNP-ID	extension primer
rs1334894	TTGATGACTTAGTCCTGTC
rs1360780	GGCTTTCACATAAGCAAAGTTA
rs15258	TTTAATACACTATTGGATTTTTT
rs1546428	AATATGCCTCCTGGCGTT
rs1802039	AGCAGAAAAAGGCTGTGCTGCC
rs1803817	CTATGCTTCTGTCTCCAC
rs1903370	TCCTTCCAGGCTTTTCTTTAG
rs1981656	CAAGAGAGAGGGCACAGGT
rs2118727	AAAGTATGCTACCGGAAACAC
rs2290893	GTAAATTATGAGACAAACTTTT
rs258813	CTAACTACAGTGATTTTGTC
rs2740204	TGGGTGTCTGTGGCTCT

rs2958153	GTTACCGAAAGAGGCGAGTA
rs2958154	AGCTTGTCATTTCTCACCTTT
rs3021522	GAGTCACGTACAGGGTG
rs3176921	AGCCTGCAGAAGCAAGGCCAATAA
rs3204090	GTTATTGACGCATTCATCTCTGA
rs33388	ATGCTTCTCTAGGTGTGTGA
rs33391	CCTCTGAAAATCCTGGTAA
rs5195	GCTGCCAGGAGGAGAACTACCTGC
rs6156	GGAAGACAACCTCCAGAG
rs6157	CAGAGAGACGTCTCCGG
rs6158	GCGCTTCGCAGGTGAGC
rs6159	CTCCTCGCTCCTCGCCGG
rs6188	TTACAGTTCATTTCTATGTATTT
rs6191	CTGTAGGTGAATGTGTTTTT
rs6192	GCGAAGACTTTTGGTTGAT
rs6195	TTCCCGTTGGTTCCGAAA
rs6196	ACAGAAGTTTTTTGATATTTCC
rs6597	TGGGGAAGTGTGGATGTTAGC
rs706118	TGGGCCATAGGAACTGATCT
rs706120	TCAGGACTTGCCACAAAGAGAA
rs734369	AAGACTGCAGATCTCCATGTGCCA
rs737054	GACGCCCAGGCACAGCC
rs755658	GCGCGTACATCTCACTG
rs2103681	ATCTATTTCTGTAAAACTCAG
rs1883636	CCCACAGTTGTCCTTCC
rs1540910	CTTCCCTGGAAACCCCAAG
rs4713878	GACAAGTTACTTAACTTCTCTGAG
rs1883637	CCACTAGAGCCCCATAATTTCTC
rs992105	TCACCTTGTATTTCTAAAGAT
rs1051952	TCCGGGAGCAGTAGTCA
rs2273000	GGGTGGAGGCATATATTGGTCT
rs4713897	TTCTGGAACATAATGTGAGC

rs734369	GCAGATCTCCATGTGCCA
rs747411	CTATGCTGTCCTGGCTGGTCTC
rs1320991	GCGTATGCATACTGTGTGTATA
rs737054	GACGCCCAGGCACAGCC
rs3777747	ATTCCTCTCCTTTCCAGC
rs4401662	GAGCTGCGATAGCATCACT
rs4713908	TGTTTTCTTTACTCATTTAACTTA
rs755658	GCGCGTACATCTCACTG
rs4713899	AGGCTCATCTGCATCTGTTAC
rs1360780	AGGCTTTCACATAAGCAAAGTTA
rs4713907	CTGTAGAATCCGTAGAATC
rs2092427	AACCATCACTAAAGAAGTCAGCAA
rs4713905	TTAGAAGATGTGAACTACATT
rs4520009	AGGAAACTGAAAGCTAAAAGTTCA
rs4713906	CCTGTAATCCCAGCATTTT
rs4713916	GACTCCTACATTTTCCTCT
rs2143404	GAGCTCAGTTCCCAGGG
rs4711429	ATCTGTAACTTAGAGCCCTT
rs9462104	CCTTAGGCATAACCACC
rs9394312	TTCTCCCGAGGCTCCCA
rs943297	CTGGACACAATTCCTTAGTTA
rs9380528	AACTGAGATCATGCCACT
FKBP5UT5A	GGAAAGGGCTGCTCATCC
FKBP5UT5C	TCATTAACGCAGAAAAACAAA
rs9380526	GAGGACAGACAAATGACT
rs10947563	CAGTAATATGCTTGCTGTACC
rs9380525	TTACTAGTGACATTCTAAAGGAG
rs6912833	GATGCAAGAATAGCATGGATC
rs7748266	GAACAAGAAACCAGTTGTATCA
rs9470069	CTGTAATTCCAGCACTTTG
rs6926133	AGTCTTTAAGTTTAATTGCAGGTC
rs2395634	TAGAATGTTCTTACACAAATCTAG

rs7753746	GTGTTTCTGGTTTGTTACC
rs10807151	TGGCAGAACAATGCATC
rs9348978	CTGAGCAGGTGAAAAAT
rs11751447	GGTGCCTCGTGCAGAGC
rs2395631	TTTCATCTGATAGGCCCAAC
rs4713902	CATGAAGAGCTAATTCCTTTATCA
rs1334894	GGAAGTTGATGACTTAGTCCTGTC
rs1475774	CTTGTTTTCTAATGATTCAAG
rs2817035	GGCTCCCTACTCCACCACTAC
rs3807050	CCAGTAAAGACTTGCCTGA
rs1977655	TCTGCTAAAAATACAAAAATAGCC
rs3800374	CCCATTTTACAGTTATGGGCTC
rs4713912	GCCACCCGAGAAGCTGGGATTA
rs2817047	CCCCCCAAGTAGCTGGGACTACA
rs4713913	GTGTTTTTGTTTGTTTGTTTGTTT
rs4713921	CAGTTGAAAGAGCCTCAC
rs2817041	GTGCAGGATCATGCTCTTGGC
rs2766543	CTTTTCTCCCTGCTACGT
rs5020575	AGTGATTCTCGTGCCTCA
rs1591365	TGTCAGTTGTTTTTCCTTGAA
rs2296662	CTGGGCTCGCTTCCCTCCC
rs2817010	AGAAGCAGCCTATGTTGAGG
rs873941	TTTTGCAACTCACTTTTTAAAA
rs3800373	AAGAGCAACTATTTATTTGTCAAC
rs4713903	CCAAACAACTAAATTGGGAAA
rs2766534	CCGCCCTACACTTTCAC
rs2766554	CTTTTGCCCTCACATTCTT
rs2817054	AGCAGCGGCTCAGGCAG
rs2766597	AGAAGATCCTGATCCTCC

Neuroendocrine assessment using the combined dexamethasone suppression/CRH stimulation (Dex-CRH) test:

For controls and remitted patients, Plasma was collected at the same timepoint as blood was drawn for protein or mRNA extraction to measure cortisol plasma concentrations. For depressed patients, the Dex-CRH test was administered to 241 patients of the Max-Planck Institute of Psychiatry on average within the first 7.2 days of admission (SD = 3.2). The Dex-CRH test was performed as described in detail in Heuser et al., 1994[18]. Patients were administered the test within the first ten days of admission (n= 225) and the last ten days of discharge (n = 150). Briefly, patients were pre-treated with 1.5 mg of dexamethasone per os at 23:00. The following day a venous catheter was placed at 14:30 and blood was drawn at 15:00, 15:30, 15:45, 16:00 and 16:15 into tubes containing EDTA and trasylol (Bayer Inc., Germany). At 15:02 100µg of human CRH (Ferring Inc., Kiel, Germany) was administered intravenously. For the area under the curve (AUC) of the cortisol and ACTH response, the area under the concentration-time course curve corrected for the baseline value at 15:00 was computed using a trapezoidal integration for the test at admission as well as at discharge. Hormone assays for the Dex-CRH test were identical to those described in detail in Zobel et al., 2001[17]. Briefly, for the measurement of plasma cortisol concentrations, a radioimmunoassay (RIA) kit from ICN Biomedicals, Carson, CA was used where the detection limit was 0.3 ng/ml. For plasma ACTH concentrations an immunometric assay without extraction (Nichols Institute, San Juan Capistrano, CA) was used, with a detection limit of 4.0 pg/ml.

Quantification of FKBP51 protein levels in lymphocytes:

T cells were extracted from the whole blood of healthy controls using magnetic beads with antibodies against CD2 (Dynal Inc.). Cells were then lysed in a protein lysis buffer. Protein levels of FKBP51 were assessed using Western-blot analysis.

For protein extraction cells were solubilized in 1ml lysis buffer (20 mM Tris/HCl pH 7.5; 130 mM NaCl; 20 mM Na_2MoO_4;1 mM EDTA; 10% glycerol; 0.5% Triton 100; 1:100 protease inhibitor cocktail P2714 from Sigma). The extract was incubated for 1 h on ice, centrifuged for 4 min at 13000 rpm and protein concentration was determined.

An alternate method for evaluating FKBP51 protein levels consists of extracting Lymphocytes from whole EDTA blood of healthy controls and remitted depressed

patients using magnetic beads labeled with anti-CD2 antibodies according to standard protocols (Dynal Inc.). Briefly, EDTA blood was incubated with 100µl anti-CD2 Dynabeads for 20 minutes. Lymphocytes were then isolated using a magnetic device and 3 washes with PBS containing 2% fetal calf serum. Lymphocyte isolation and protein extraction occurred immediately following blood draw. For protein extraction cells were then dissolved in 250µl lysis buffer (20 mM Tris/HCl pH 7.5; 130 mM NaCl; 20 mM Na_2MO_4; 1 mM EDTA; 10% glycerol; 0.5 % Triton 100; 1:100 protease inhibitor cocktail P2714 from Sigma). The extract was incubated for 1h on ice, centrifuged for 4 min at 13000 rpm and protein concentration was determined using the BCA-kit (Pierce).

For immunoblot detection, 10 µg and, in an additional set of experiments, 20 µg of cell lysates' total protein were separated by SDS-PAGE under denaturing conditions. The proteins were transferred to polyvenylidene difluoride membrane (Schleicher & Schüll GmbH, Germany). Nonspecific binding to membrane was blocked by 5% nonfat milk in Tris-buffered saline/Tween buffer. FKBP51 was detected by a monoclonal anti-FKBP51 antibody (Stress Gen Biotech) followed by horseradish peroxidase-conjugated rabbit anti-mouse antibody (Sigma). Signals were visualized by ECL solution (Amersham Pharmacia Biotech) and monitored in an imaging system (Kodak Imaging Station 440). Light emission was normalized by the intensity of a recombinant FKBP51 standard that was loaded onto each gel to ensure accurate comparison of probes loaded on different gels.

Statistical analysis:

All analyses for binary outcomes were performed using logistic regression using both R and SPSS (version 11), as well as by exact contingency table analyses using SPSS. Quantitative variables were analyzed using logistic regression analysis. Linkage dysequilibrium was calculated using the "Gold" software.

Appropriate correction for multiple testing in association studies with markers in linkage disequilibrium (LD) was done by performing different methods of correction and their results.

1. The classical Bonferroni correction. Here we have a total of 59 polymorphic SNPs and four phenotypes (depression per se, response at 2 weeks, at 5 weeks and at time of discharge). This gives a total of 59*4 = 236 tests.

2. A method recently described by Nyholt[28], which aims at estimating a number of effective tests by taking into account intermarker LD. This reduces the number of effective tests by roughly a factor 2, thus leading to a number of effective tests of 118.

3. permutation based methods, taking into account

a. the correlation between the response phenotypes, effectively creating a joint response phenotype analysed.

b. the LD between SNPs

c. LD between the SNPs and the correlation between the phenotypes.

The p-values for methods a, b, and c were obtained using 50000 replicates each for methods b and c and 500,000 replicates for method a, using the method of Ge and colleagues (http://personales.unican.es/cofinoa/test/). Taking into account dependencies between SNPs and phenotypes was done using Fisher's product method.

For method a) the nominal p-value was 0.000036 for SNP rs3800373 with the joint response phenotype. This has to be Bonferroni-corrected by the fact that we are dealing with two phenotypes now (depression per se and the joint response phenotype) and have looked at 8 genes, with 32 SNPs typed in and around the FKBP51 gene, which also contains the rs3800373 SNP. Thus the corrected p-value for this SNP is 0.000036 * 2 * 8 * 32, which is equal to 0.018432.

For method b, we have a nominal p-value of 0.00018 for response at two weeks and the jointly considered SNPs in FKBP5, which needs to be multiplied by a factor of 3 to arrive at a p-value of 0.00054 to adjust for the three response phenotypes. This value of 0.0054 needs to be corrected again by a factor of 16 (8 genes and 2 phenotypes (depression per se and response) to arrive at a final p-value of 0.00864.

Finally for method c, which uses a Fisher product of p-values acroos all SNPs in the FKBP51 region and across all phenotypes, we arrive at a nominal p-value of

0.00014. This again has to be corrected for by a factor of 16 (8 genes and 2 phenotypes) to arrive at a corrected p-value of 0.00224.

Recent literature, advocating (haplotype-) block-wise significance tests[29] or gene-wide significance tests[30], which in our case are equivalent, that method c is actually the most appropriate method of analysis. However, we present all these results for the reader's perusal.

To control for stratification we used the genomic control approach of Devlin and Roeder (1999), with the estimation of the stratification parameter λ performed using the likelihood method of Freedman[31]. We used 100 unlinked SNPs to perform the analysis.

In addition we also performed one-way and repeated measures analysis of variance (ANOVA), a stepwise (forward inclusion) multiple regression analysis and correlation analysis using SPSS.

Individual haplotype assignments were determined using SNPHAP (http://www-gene.cimr.cam.ac.uk/clayton/software/snphap.txt). Only haplotype assignments with a remaining uncertainty of less than five percent and haplotypes with a frequency over five percent were included in the analyses. Removing these restrictions turned out not to alter our conclusions, with p-values showing only minimal changes. To test for the simultaneous involvement of several polymorphisms we also applied the method of Cordell and Clayton[32]. For the analysis of the LD pattern and haplotype block delineation we used haplotypes estimated by PHASE (http://www.stat.washington.edu/stephens/phase.html, [33]) in our sample of controls as input to HAPLOBLOCKFINDER (http://cgi.uc.edu/cgi-bin/kzhang/haploBlockFinder.cgi/, [34]). Haplotype block definition was done using the |D'| method[35] with a threshold of 0.75. SNPs with a frequency of the minor allele less than 0.1 were omitted from the analysis. We also used the R package "genetics" (http://lib.stat.cmu.edu/R/CRAN/) to compute pairwise D' values from genotype data for a graphical depiction of LD via GOLD (http://www.sph.umich.edu/csg/abecasis/publications/10842743.html, [36]).

Example 2

Association study of polymorphisms in FKBP51 and response to antidepressant drugs

30 SNPs in FKBP51 (see table 1) were genotyped in 327 patients with major depression. 103 of them showed an improvement of over 25% in disease severity as measured by the Hamilton Depression Rating Scale (HAM-D) after two weeks of treatment with an antidepressant (response), while 156 (non-response) showed less than 25% improvement. The patient population used for the studies comprises of individuals that were treated with various antidepressant drugs including, Amitriptylin, Amitriptylinoxid, Clomipramin, Doxepin, Imipramin, Nortriptylin, Trimipramin, Citalopram, Fluoxetin, Fluvoxamin, Paroxetin, Sertralin, Tianeptin, Mirtazapin, Venlafaxin, Reboxetin, Moclobemid, Tranylcypromin, Bupoprion, Buspiron, Dibenzepin, Nefazodon, Opipramol, Sibutramin, and Trazodon. Most patients received only one of these antidepressants as monotherapy regiment, but some individuals received combination therapy (i.e. more than one antidepressant).

We could show a significant association of 30 SNPs in FKBP51 with response after 2 weeks of treatment.

Table 8: Association of SNPs in FKBP51 with response at 2 weeks.

SNP	Non-responders (n = 156)	Responders (n = 103)	p-value
rs2766534	GG:4.6%, GT:44.5%, TT:50.8%	GG:2.2%, GT:28.9%, TT:68.9%	0,0057
rs4711429	AA:3.4%, AG:40.7%, GG:55.9%	AA:14.0%, AG:35.5%, GG:50.6%	0,0076
rs4713921	CC:54.7%, TC:42.2%, TT:3.1%	CC:49.4%, TC:36.7%, TT:13.9%	0,0036
rs9462104	CC:2.6%, TC:39.5%, TT:57.9%	CC:13.5%, TC:35.6%, TT:50.9%	0,0043
rs9394312	CC:24.6%, GC:61.0%, GG:14.4%	CC:27.3%, GC:42.4%, GG:30.2%	0,0016
rs4713916	AA:1.0%, AG:41.0%, GG:58.0%	[illegible], AG:33.6%, GG:50.8%	5.5×10^{-5}
rs943297	AA:1.7%, AG:42.0%, GG:56.3%	AA:14.5%, AG:35.8%, GG:49.7%	3.1×10^{-4}
rs9380528	AA:21.8%, AG:61.3%, GG:16.8%	AA:24.6%, AG:42.7%, GG:32.7%	0,0034
FKBP5UT5A	GG:99.99%, GC:0.01%, CC:0.0%	GG:99.69%, GC:0.4%, CC:0.01%	-
FKBP5UT5C	AA:0.0%, AG:0.0%, GG:100.0%	AA:0.0%, AG:0.01%, GG:99.99%	-

rs9380526	CC:3.4%, TC:45.8% TT:50.8%	CC:17.4%, TC:36.0% TT:46.5%	4.8×10^{-4}
rs10947563	AA:61.5%, AG:36.5%, GG:2.1%	AA:54.2%, AG:29.9%, GG:16.0%	0,0013
rs9380525	CC:50.8, GC:46.6, GG:2.3%	CC:47.6, GC:35.9, GG:16.5%	2.7×10^{-4}
rs6912833	AA:1.9%, TA:47.7%, TT 50.5%	AA:16.2%, TA:42.6%, TT 41.2%	3.5×10^{-4}
rs2143404	CC:74.8%, TC:25.2% TT:0.0%	CC:71.1%, TC:25.4% TT:3.5%	0,046
rs1360780	TT:1.0%, CT:40.8%, CC:58.2%	TT:14.7%, CT:35.9%, CC:49.3%	1.2×10^{-4}
rs1591365	AA:55.5, AG:42.2%, GG:2.3%	AA:51.5, AG:34.4%, GG:14.4%	6.2×10^{-4}
rs7748266	CC:75.2%, TC:24.8% TT:0.0%	CC:71.7%, TC:24.3% TT:4.0%	0,0348
rs9470069	CC:0.8%, GC:25.2%, GG:73.9%	CC:0.0%, GC:15.6%, GG:84.4%	0,045
rs6926133	AA:0.0%, AC:27.4%, CC:72.9%	AA:5.3%, AC:24.6%, CC:70.2%	0,0135
rs3777747	AA:18.8%, AG:61.7%, GG:19.5%	AA:33.9%, AG:43.9%, GG:22.2%	0,0061
rs4713899	AA:0.0%, AG:24.2%, GG:75.8%	AA:3.3%, AG:26.7%, GG:70.0%	0,0244
rs2395634	AA:2.5%, AG:41.5%, GG:55.9%	AA:13.3%, AG:34.7%, GG:52.0%	0,0036
rs7753746	AA:73.9%, AG:26.1%, GG:0.0%	AA:72.7%, AG:23.3%, GG:4.1%	0,0328
rs3800373	CC:0.0%, CA:37.9%, AA:62.1%	CC:13.5%, CA:33.8%, AA:52.7%	2.8×10^{-5}
rs10807151	CC:0.0%, TC:23.5% TT:76.5%	CC:5.2%, TC:23.1% TT:71.7%	0,0125
rs3800374	CC:75.0%, TC:24.2% TT:0.8%	CC:65.4%, TC:29.6% TT:5.0%	0,0354
rs9348978	AA :11.3, GA :58.3, GG :30.4	AA :30.1, GA :48.0, GG :22.0	5.8×10^{-4}
rs11751447	CC:18.6%, TC:55.9% TT:25.4%	CC:33.3%, TC:46.8% TT:19.9%	0,0179
rs2395631	AA:17.9%, AG:57.3%, GG:24.8%	AA:33.5%, AG:47.6%, GG:18.8%	0,0114

This effect, however, was not limited to response at 2 weeks, but could be observed during the whole in-patient treatment course. This is illustrated with rs1360780 in Figure 3a, b, c, d, with rs4713916 in figure 6a and with rs 3800373 in Figure 7a.

All 30 SNPs genotyped in FKBP51 are in strong linkage dysequilibrium (see figures 1 and 2). This implies that the causal variant could lie anywhere within the linkage disequlibrium block defined by these SNPs.

We first analyzed 57 SNPs in these genes in 294 depressed patients and 339 healthy controls, matched for age, sex and ethnicity. Haplotypes were reconstructed for all SNPs within a gene. After correcting for multiple testing, we could not detect any significant differences in frequencies of single SNPs or haplotypes between our patient and our control sample. We then tested all single SNPs and haplotypes for an association with parameters related to the response to antidepressant drug treatment after 2 weeks and 5 weeks of hospitalization and to remission of symptoms at discharge (N = 233 depressed patients). There were significant associations between three SNPs in the FKBP51 gene and response at at least two of the three time points. For rs1360780 the nominal p-values for association with response at 2 weeks and 5 weeks and remission at discharge were 0.00048, 0.0027 and 0.092 respectively. For rs1334894 they were 0.050, 0.0054 and 0.030; and for rs755658 they were 0.036, 0.010 and 0.043.

We therefore focused on the region of FKBP51 and genotyped an additional 27 SNPs in 288 kb around this gene to investigate whether this result could indeed be attributed to FKBP51 and not adjacent genes. The region investigated included the neighboring genes TULP1 (3' of FKBP5), the hypothetical protein FLJ25390 and CLPS (both 5' of FKBP5), with an average distance of 9.6 kb between SNPs. Using genotype data from the control group, we constructed a linkage disequilibirium (LD) block map based on D' for this region. We detected a single large block of LD that encompasses most of the FKBP51 gene plus a region 5' of the gene, with the block ending before FLJ25390. In addition, four minor blocks of LD are detected by HAPLOBLOCKFINDER in our data (see figure 1). We then tested all SNPs in this region for association with response to antidepressant treatment. For the association with response at 2 weeks, the smallest p-values were found with SNPs within the major block of LD containing FKBP51 (see figure 2). Here we observed 3 peaks of strong association, one at the 5' end of the gene with rs4713916 (5' of FKBP5, potential promoter region, nominal p = 0.00031), the second in intron 2 (rs1360780, nominal p = 0.00048) and the third in the 3' untranslated region of FKBP51 (rs3800373, nominal p = 0.00003). A similar pattern of association was found with response at 5 weeks and remission. Adjustment for multiple tesing was performed

using various methods, all showing that the observed association was significant after adjusting for multiple testing. The classical Bonferroni correction gave a corrected p-value of 0.00708, the method recently described by Nyholt[28] a corrected p-value of 0.00354, both for rs3800373 and response at two weeks. Permutation based methods, taking into account the correlation between the response phenotypes, effectively creating a joint response phenotype analysed (a), the LD between SNPs (b) and LD between the SNPs and the correlation between the phenotypes (c) gave corrected p-values of 0.018432 for rs3800373 and the joint response phenotype (a), 0.00864 for an association of all the genetic variation in and around FKBP51 considered and response at two weeks (b), and 0.00224 for an association of all the genetic variation in and around FKBP51 considered and the joint reponse phenotype (c), respectively.

The clustering of significant associations within the LD block containing FKBP51 indicated that the observed associations can be attributed to this gene. It is, however, not clear which one of the highly associated SNPs could be the causal variant or might be closest to it as all three of them are in strong LD (D' > 0.8). Both haplotype analysis and the stepwise procedure of Cordell and Clayton[32] did not hint at untyped causal polymorphisms, most likely because of the high degree of LD across all of FKBP5.

Example 3

Association of rs1360780 genotype with HPA-axis activity

To investigate changes of HPA-axis activity from admission to discharge, we used the Dex-CRH test, which sensitively identifies GR-signaling impairment[18]. By comparing the ACTH and cortisol responses in this test of patients with CC/CT or TT genotypes of rs1360780, we observed that at admission T homozygotes displayed a significantly lower ACTH response following CRH stimulation than patients with the CC or CT genotypes (figure 4). Similar results were obtained with rs4713916 and, rs3800373 (figures 6b, 7b). An elevated ACTH response in this test indicates an impaired negative feedback through GR. Our finding thus suggests that, even though T homozygotes are as severely depressed as patients with the two other rs1360780 genotypes, their GR sensitivity is not as much impaired, allowing a faster restoration

of normal HPA-axis function by antidepressants and thus an accelerated clinical response.

We plotted the weekly scores of the Hamilton Depression Rating Scale (HAM-D), measuring the severity of depressive symptoms for the first five weeks of treatment against the rs1360780 (figure 3a), rs4713916 (figure 6a, d) and, rs3800373 (figure 7a, d) genotypes. For rs1360780, repeated measures analysis of variance (ANOVA) showed T homozygotes to have an accelerated response over the whole time course ($p = 0.00015$ for genotype effect), while the response of patients with the CT and CC genotypes was significantly slower and almost identical. Overall, duration to response, as defined by a reduction of the HAM-D scores from admission by more than 50%, was significantly shorter for T homozygotes with 3.18 weeks (SEM=0.62) against 5.03 weeks (SEM=0.244) for heterozygotes and C homozygotes combined ($p = 0.006$).

Differences in response to antidepressant drugs among groups of patients can be due to a series of confounding variables, including population stratification. To exclude the latter possibility, we estimated the amount of stratification in responders and non-responders. To this end we genotyped a sample of 100 unlinked SNPs. We analysed the data using the likelihood method of Freedman[31]. The estimate of λ_{ll}, the parameter measuring the population stratification, is 1.000, with the upper bound of the 95% confidence interval being at 2.081. It should be noted that this upper bound is the upper bound for $\tilde{\lambda}_{ll}$For the most significant p-value of 0.00003 obtained with rs3800373 and response at two weeks (obtained with 96 non-responders and 146 responders), this upper bound translates into an upper bound for $\lambda_{96|46}$of 1.125, owing to the linear relationship between sample size and λ described by Freedman et al. (2004). Therefore, to take an example, the p-value obtained using the classical Bonferroni correction (0.00708) cannot be reasonably due to population stratification. Taking this p-value as an example and applying the correction described by Freedman[31] (for which we transform the p-value obtained by logistic regression into a chisquared variable with one degree of freedom (7.253)), the p-value for a chisquared variable with one degree of freedom of 7.253/1.125 is 0.011, clearly significant.

Besides population stratification, differences in treatment regimen, in sex or age, in disease subtypes or the presence of psychiatric and somatic comorbidities could confound our results. To exclude the possibility that stratification in these variables

(age, sex, type of antidepressant medication, presence of adjunct mood stabilizer, benzodiazepine or antipsychotic treatment, length of antidepressant treatment before admission, melancholic vs. non-melancholic, bipolar vs. unipolar and psychotic vs. non-psychotic depression or the number of psychiatric and somatic comorbidities) was responsible for the observed effects, we included these 12 variables together with the genotype of rs1360780 in a regression analysis with response at 2 weeks as the dependent variable. For antidepressant medication, we grouped patients according to the primary class of antidepressant in patients treated with selective serotonin reuptake inhibitors (N = 70), tricyclic antidepressants (N = 48) and patients treated with mirtazapine (N = 55), an antidepressant mainly targeting serotonin 2C and alpha2A-adrenergic receptors. Patients receiving a combination of antidepressants from more than one class of antidepressants were included in a fourth group. In this stepwise regression analysis containing the above-named 13 variables, only the rs1360780 genotype and length of antidepressant treatment before admission were included as significant in the model while the remaining nine variables were not found to contribute significantly to the phenotype. The longer patients had been treated in an out-patient setting before admission, the less likely they were to be in the early responder group. The inclusion of this variable did, however, not influence the significant association of rs1360780 with response. This suggests that the observed association of FKBP51 polymorphisms with response to antidepressant treatment is not likely to be due to stratification in these other clinical variables, including differences in the class of administered antidepressant. Almost identical results were obtained in the three primary antidepressant treatment classes for the association of rs1360780 with response to antidepressant treatment (see figures 3b, c, d), suggesting that this effect is independent of the primary pharmacological profile of antidepressants. This supports the notion that FKBP51 is involved in the common final signaling pathway of antidepressant drugs.

We then investigated whether the rs1360780 genotype was associated with other disease-associated variables than response to antidepressant treatment. No genotype-dependent differences in sex, age, age of onset or the diagnostic subgroup (unipolar, bipolar, psychotic, presence of melancholic features) of depressed patients were observed. There was, however, a significant difference in the number of previously experienced depressive episodes ($F_{282,2} = 5.3$; $p = 0.005$; ANOVA with age as covariate). Patients with the TT genotype of rs1360780 experienced over

twice as many depressive episodes (see figure 5) before the index episode suggesting these patients have more lifetime depressive episodes in addition to their faster response to antidepressant drugs. Similar results were observed with rs4713916 and, rs3800373 (figures 6c, 7c)

Example 4

Association of rs1360780 genotype and FKBP51 protein expression in lymphocytes

Polymorphisms in *FKBP51* could influence GR sensitivity by several means. Polymorphisms in regulatory regions could lead to an altered expression level of FKBP51, varying the stoichiometric proportions of FKBP51 and FKBP52. Polymorphisms leading to amino acid exchanges may change the affinity of FKBP51, modifying its exchange rate against FKBP52. Both types of mutations could alter the rate of nuclear translocation of the activated GR. Glucocorticoids induce FKBP51 expression via an ultra-short negative feedback loop for GR activity[24]. Polymorphisms decreasing the induction of FKBP51 expression by glucocorticoids could thus lead to a maintained GR sensitivity despite high circulating cortisol levels, resembling somewhat the neuroendocrine situation observed in T homozygotes. In order to investigate whether the genotype of rs1360780 was associated with different levels of FKBP51 protein, we quantified this protein in lymphocytes (T-cells) of healthy probands (Figures 8, 9). Lymphocytes of individuals homozygous for the T allele of the rs1360780 SNP, the genotype associated with a fast response to antidepressant drugs, have FKBP51 levels that are twice as high as those of individuals with the CC or CT genotypes ($p < 0.05$). Semi-quantitative analysis of Western blot results (see Figure 8) revealed that probands homozygous for the CC or CT allele of rs1360780 showed the lowest FKBP51 protein levels in lymphocytes (see Figures 8, 9). Higher levels of FKBP51 thus could be associated with an enhanced GR sensitivity that would prevent long-lasting dysregulations of the HPA-axis (see Figure 4) and in consequence a faster response to antidepressant drugs (see Figure 3a,b,c,d).

To identify the mechanism of increased FKBP51 protein levels, we quantified FKBP51 mRNA levels in peripheral blood monocytes (PBMCs) of these probands. There were no direct effects of the rs1360780 genotype on mRNA levels (figure 10),

suggesting that not increased transcription but enhanced translation or protein stability contribute to the increased FKBP51 levels. We did, however, observe genotype-dependent differences in the positive correlation of plasma cortisol and FKBP51 mRNA levels. As noted above, glucocorticoids have been shown to induce FKBP51 expression *in vitro*[24]. This finding could be confirmed in the overall sample *in vivo* (figure 11A), where plasma cortisol and FKBP51 mRNA levels showed a significant positive correlation ($R = 0.262$, $p = 0.003$). Intriguingly, when this analysis was repeated with the sample split in probands with the TT vs. the CC and CT genotypes of rs1360780, a much stronger correlation was observed in T homozygotes ($R = 0.619$; $p = 0.003$) that was significantly different ($p = 0.012$) from the correlation observed in the other genotypes (figures 11B and 11C).

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